Summary Report

On

Formulation development of Nano Structured Lipid Carrier of Lurasidone hydrochloride for intranasal delivery

To be submitted

To

Research Advisory Committee

of

Ph.D Programme

In

Pharmaceutical Sciences

Name of the student

Ms Tanvi Kadam

Name of the guide

Dr Saritha Shetty

SPP School of Pharmacy & Technology Management

SVKM's Narsee Monjee Institute of Management Studies,

V.L. Mehta Road, Vile Parle (W.), Mumbai – 400 056.

INTRODUCTION

Intranasal administration is a non-invasive approach preferred for potent drugs with no or low oral bioavailability. Systemic, local and central nervous system (CNS) drug administration is possible through intranasal route. Drugs used to treat CNS disorders can be permeated directly via the nasal mucosa to the brain and spinal cord through the olfactory and trigeminal nerves pathways. This also minimizes systemic exposure of the drugs thus reducing their side effects. Systemic delivery of drugs to the CNS is not effective for nearly 100% large molecule and > 98% of small molecule drugs due to blood-brain barrier and (BBB) and blood cerebrospinal-fluid barrier (BCSFB) present in the brain. These tight junctions segregate the brain from circulating blood thus preventing entry of any macromolecules and drug into the brain. Consequentially several invasive strategies like intra parenchymal, intraventricular, intrathecal delivery (BBB disruption) and non-invasive techniques like prodrug approach, chemical modifications and drug conjugation with antibodies or ligands have been used to increase the CNS targeting of drugs (1).

Nano structured lipid carriers (NLCs) are modified second-generation innovative lipid nanoparticles used as a bioactive carrier system**.** NLCs are formulated using excipients like biocompatible solid lipid matrices and liquid lipids with different chemical structures than solid lipids. NLCs can be prepared using various methods like high pressure homogenisation (, solvent emulsification and evaporation technique, high shear homogenization, ultrasonication method, solvent emulsification and diffusion technique, phase inversion or melt dispersion method and double emulsification method.2

Various types of NLCs are reported like amorphous (structure less solid amorphous matrix), imperfect (imperfectly structured solid matrix), multiple oil in fat in water (O/F/W). NLCs have several advantages, including greater physical stability, ease of manufacture and scale-up, and increased productivity due to high trapping of lipophilic and hydrophilic drugs in aqueous medium. Various emulsifiers and lipids Ingredients may be used in formulation of NLCs (2).

Process drift refers to an unexplained, unintentional or unexpected deviation of measured process parameters or resulting product attributes from their intended target values over time. It occurs as a result variations in various process inputs such as raw materials manufacturing personnel, and processing conditions or machine interactions. When effective systems are not

implemented and tools are not used to prevent these process drifts, it can lead to the manufacturing issues, including reduced product yield, delays in batch production, quality of the ingredients and packaging materials may vary. Batch failures, clinical failures related to product quality, investigations, recalls, product seizures, legal action and regulatory interventions might also be observed during product development (3). By using tools and various approaches like process analytical technologies (PATs), quality by design (QbD), *in vitro –in vivo* correlation (IVIVC), and comprehensive characterization of excipients will enhance the robustness of the finished products and minimize or prevent unintended drift in the quality of the affected commercial drug products (4-5).

Knowledge about the relationship between independent and dependent variables in formulation development trials will facilitate the product development stage. There is a need of economical and efficient method known as Optimization phase for achieving this target. Optimization has been gaining popularity in pharmaceutical research, day by day, since the best results can be obtained in a limited number of experiments (6).

Nano drug delivery, although seems to be best in delivery aspects but at the same time presents lot of challenges in the development programme. Hence, the study was undertaken develop a nano formulation of Lurasidone for nose to brain delivery.

RATIONALE

Psychotic disorders are one of the main public health concerns, affecting a significant section of populations around the world. Amongst these, Schizophrenia is one of the psychotic disorders that has been identified as one of the most disabling health conditions. In the year 2021, the schizophrenia market size was $9.0 billion and is expected to achieve a CAGR of more than 3% during 2021-2031. [https://www.globaldata.com/store/report/schizophrenia-market-analysis/]

Despite the critical function of antipsychotic drugs in the treatment of psychotic disorders, these drugs have considerable drawbacks**.** Extrapyramidal side effects and, more rarely, tardive dyskinesia occur in a small percentage of patients. Another rare but possibly life-threatening side effect of antipsychotic medication is neuroleptic malignant**.**

Antipsychotic medicines operate on the brain's receptors in a variety of ways. One of the most impenetrable barriers to medicated molecules entering the brain is the blood-brain barrier (BBB). A variety of approaches has been applied to deliver drugs directly to the brain. However, nose-to-brain delivery to transfer therapeutic medications to the brain has gained a lot of interest in recent decades since it has a lot of benefits for drug introduction.

Lurasidone hydrochloride (LH), benzisothiazole derivative, was approved for the treatment of schizophrenia in the United States in October 2010 and in Canada in June 2012**.** The reference listed drug commercially available is an oral tablet of Lurasidone formulation with the brand name Latuda®, which was sold all over the world. Lurasidone hydrochloride has poor bioavailability of only 9-19 % after oral administration of 40mg tablet. Cmax of 53ng/mL is reached in 2.0hours [1-2]. Owing to its poor bioavailability a technique that increases bioavailability by targeting the receptor site and bypassing the BBB is needed to obtain enough drug concentration for effective therapeutic action.

Intranasal delivery to brain prevents first pass metabolism and reduces side effects and increased onset of action. Intranasal delivery could also circumvent the BBB for better targeting of the drug to the brain. Nanotechnology based delivery systems, due to smaller size, hold great potential for delivering drugs to brain when given intranasally**.** Nano drug delivery systems, with particle sizes in nm can directly bypass blood brain barrier and can prevent passage of drug into lungs which can lead to the draining of drugs from the nasal cavity**.** Thus, intranasal delivery of Nano structured lipid carriers can deliver drugs more effectively for give quick onset of action.

For the drug to be absorbed from nasal cavity, it depicts many advantages like large surface area, porous endothelium basement membrane, highly vascularized epithelial layer, high total blood flow per cm3, avoids first pass metabolism and easy access to CNS

Therefore, it is the most - feasible and non-invasive route for enhancing bioavailability of LH and achieving brain targeting.

Intranasal delivery to brain prevents first pass metabolism and reduces side effects and increased onset of action. Nano drug delivery due to particle size in nm can directly bypass blood brain barrier and prevents passage of drug into lungs and getting drained from the nasal cavity.

Nose to brain delivery of Lurasidone NLC will give quick onset of action. Also, NLC will give steady state release of drug in brain.

Graphical abstract for formulation development of nanostructured lipid carrier of Lurasidone hydrochloride is shown in Figure 3.1.

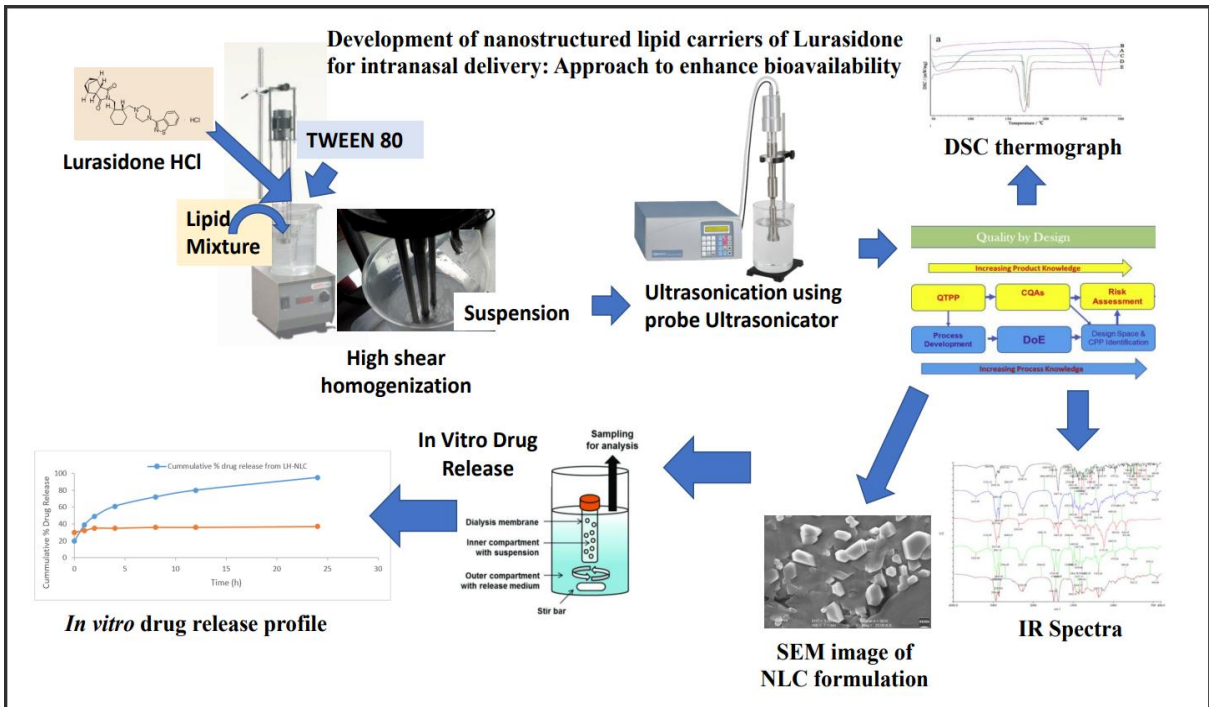


Figure 3.1 Graphical abstract for formulation development of nanostructured lipid carrier of Lurasidone hydrochloride

AIM: To Optimize the formulated Nano Structured Lipid Carrier of Lurasidone hydrochloride for intranasal delivery.

OBJECTIVES:

- To carry out the pre formulation studies for appropriate selection of excipients.
- To optimize and formulate Lurasidone HCl loaded Nano Structured Lipid Carrier (LHNLC) using 3^3 Box Behnken design.
- To perform physicochemical characterization and stability studies of optimized LHNLC batch.

- To develop an analytical method for the estimation of drug from developed formulation.
- To develop bio analytical method for the estimation of drug from developed formulation in blood plasma.
- To perform in vitro, ex vivo, in vivo pharmacokinetic and brain distribution studies for evaluating the diffusion, bioavailability, brain biodistribution of the optimized Lurasidone HCl NLC formulation.
- To perform histopathology study on to check safety of LH-NLC on nasal cavity

PLAN OF WORK

1. Literature and Patent search
2. Procurement of API & excipients
3. Characterisation of API and excipients as per monograph as applicable
4. Pre-formulation studies
5. Placebo trials
6. Analytical method development using stability indicating force degradation studies.
7. Bio analytical method development and validation
8. Development of formulation
9. Evaluation of the batches
10. Optimisation of trials using Design of Experiment (DOE)
11. Scale-up batches and their evaluation
12. *In vitro* and *ex vivo* studies
13. Stability studies
14. Histopathology study
15. Pharmacokinetics studies
16. Bio distribution studies
17. Communication for publication.

TIMELINES FOR THE PROPOSED WORK

Activities and timelines for completing the same is mentioned in Table 3.1

Table 3.1 Plan of work with timelines

Plan of Work	**Activities completed status**									
	3M	6M	8M	12M	24M	28M	36M	48M	60M	72M
Literature and Patent search										
Procurement of APIs & excipients										
Characterisation of API and excipients as per monograph as applicable										
Pre-formulation studies										
Placebo trials										
Bio analytical method development and validation										
Formulation development										
Evaluation of the batches										
Optimisation of trials using Design of Experiment (DOE)										
Scale-up batches and their evaluation										
Stability studies										
Invitro, exvivo and histopathological studies										

Plan of Work	**Activities completed status**									
	3M	6M	8M	12M	24M	28M	36M	48M	60M	72M
Pharmacokinetics studies										
Bio distribution studies										
Communication for publication										

LITERATURE DETAILS:

An exhaustive literature search was carried out for understanding the properties of the drug and deciding the methods for formulation development. The details are as follows:

Different CNS drugs in Nanoparticulate drug delivery for nasal drug delivery to brain.

Drug	Category	Route of administration / Dosage form	Nano drug delivery	Manufacturing Method	Ref
Ropinirole hydrochloride	Anti-Parkinson	Nasal In situ muco adhesive gel	NLC	Hot high shear homogenization method	7
Rasagiline	Anti-Parkinson	Intranasal to brain	Nanoparticulate system	ionic gelation of CG with tripolyphosphate anions	1
Pramipexole hydrochloride	Anti-Parkinson	Oral drug delivery	Chitosan nanoparticles	Ion gelation	8
Curcumin	Anticancer	Intranasal to brain	NLC	Hot high pressure homogenization	9
Glial cell-derived neurotrophic factor (GDNF)	Parkinson Disease	Intranasal to brain			10
Albendazole sulfoxide and Curcumin	Neurocysticercosis (NCC), a brain infection	Nasal to brain	Micro emulsion	Micro emulsion	11
Asenapine	Schizophrenia	Nasal to brain	NLC	melt emulsification-high shear homogenization	12
Valproic acid	Antiepileptic	Nasal to brain	NLC		13
Artemether	Cerebral Malaria	Nasal to brain	NLC	Micro emulsion method	14

Duloxetine	Antidepressant	Nasal to brain	NLC	Homogenization followed by ultrasonication	15
Lamotrigine	Antiepileptic	Nasal to brain	NLC	Solvent evaporation method	16
Diazepam , Lorazepam and Alprazolam	Insomnia	Nasal to brain	Micro emulsion	Titration method	17
Rivastigmine	Alzheimer's disease	Nasal to brain	Micro emulsion & Muco adhesive Micro emulsion	Titration method	18
Carbamazepine	Antiepileptic	Nasal to brain	Micro emulsion & Muco adhesive Micro emulsion	Spontaneous emulsification method	19
Olanzapine	Schzophrenia	Nasal to brain	Micro emulsion	Water titration method	20
Valproic acid	Antiepileptic	Nasal to brain	Micro emulsion	Water titration method	21

PATENT SEARCH

Lurasidone Hydrochloride

Patent number	**Title**	**Applicant**	**Dosage form**	**Ref**
US 9907794	Pharmaceutical compositions	Sumitomo Dainnipon Pharma CO. Ltd. Japan	Immediate released film coated tablets 20 mg-80 mg	22
US 9433620	Pharmaceutical compositions of Lurasidone	Cadilla Health Care Ltd.	Immediate released film coated tablets 20 mg-80 mg	23
US 8377995	Orally disintegrating tablets	Dainnipon Sumitomo Pharma CO. Ltd. Japan	Immediate released tablets	24

1. METHODOLOGY

1.1 Procurement of APIs & excipients

- Gift sample of Lurasidone hydrochloride API procured from Micro Labs
- Below gift samples of solid and liquid lipids procured from Gattefosse
  - Compritol 888 ATO
  - Precirol ATO 5
  - Gelucire 50/13
  - Labrafac PG

Other lipids and surfactants were purchased from Sigma Aldrich and Merck.

2. Pre formulation studies

2.1 Drug Excipient characterization

The drug was characterized by DSC and FTIR for the confirmation of melting point and functional groups respectively.

2.2 Drug Excipient Compatibility studies

- 1 :1 ratio of drug and each of the excipients
- Kept at 40ºC/75 % RH for 2 months
- Fourier-transform infrared was employed to characterize the possible interaction of LRD and the excipients.

2.3. Determination of calibration curve for drug

Wavelength determination in Methanol: the drug was solubilised in methanol. the sample was scanned from 200 to 400 nm by UV spectrophotometer. Maximum absorbance observed at 234 nm.

2.4. Solubility studies of API in Lipids

Determination of solubility of Lurasidone hydrochloride in different ratios of (drug: lipid) mixture Solid and liquid lipids. Determine QTPP (Quality target product profile) and Critical Process Parameters (CPP) and Critical Quality Attributes (CQA) (26).

For liquid lipids

Weighed 10 mg of drug in 5 ml glass vial take 2ml liquid lipid surfactant and cosurfactant. Added excess of Lurasidone HCL to liquid lipid and kept on rotary shaker for 48 hours. After 48 hrs removed and kept aside for 24 hrs and then

centrifuged it for 20 mins at 3500 rpm (microcentrifuge, Biolab BL-135D AT). 1 ml supernatant was diluted to 10 ml Methanol and absorbance checked on UV Spectrophotometer at 234 nm, with Standard plot of drug in methanol, phosphate buffer pH 5.5, 6.5 and 7.4.

2.5. Solubility in Combination lipids proposed for NLC preparation: The solubility was checked in the same manner as for liquid lipids.

2.6. Determination of Log P Value or octanol-water partition coefficient (K_{ow}) of API

In conical flask weighed 10 mg of Lurasidone HCL, added 10 ml of water and 10 ml of Octanol. Prepared these samples in duplicate as Sample A and Sample B. Kept the above sample mixtures on orbital shaker for 24 hrs. After 24 hrs removed conical flasks from shaker and poured the entire solution into separating funnel and shook vigorously and kept aside for 6 hrs, to separate the 2 layers. Separated the water layer and octanol layers in separate beakers. Withdrew 1ml from each beaker and made the volume upto 10ml with octanol and absorbance checked on UV Spectrophotometer at 234 nm, with Standard plot of API in Octanol.

3. **Formulation of Placebo Trials and with drug**

Solubility studies shows maximum solubility of drug in Glyceryl monostearate and Precirol ATO 5 solid lipid. And more in caproyl 90, cremophor RH40,Tween 80, Span 80, solutol HP, Trioleate ,oleic acid ,PEG 400, castor oil and olive oil as liquid lipids and surfactants. So we initiated NLC preparation with these lipids and surfactants. However depending on Entrapment Efficiency and particle size of NLC, the lipids and surfactants may vary.

Solid lipids and liquid lipids were melted at 65-70 ^{O} C. Aqueous solution of surfactants were prepared separately and heated at same temperature as lipid phase. Aqueous phase was slowly added to lipid phase slowly under magnetic stirrer. Homogenise using magnetic stirrer at 1500 rpm for 20 mins. The resultant emulsion is then probe sonicated for 10 mins to reduce the particle size. Batches taken with drug were also passed through High Pressure homogenisation at 5000 psi for 10 -15 cycles. Particle size, polydispersity index and Zeta potential of the NLCs were determined using Zeta Sizer.

Since particle size was achieved only using probe sonication, HPH step was omitted from finalised method

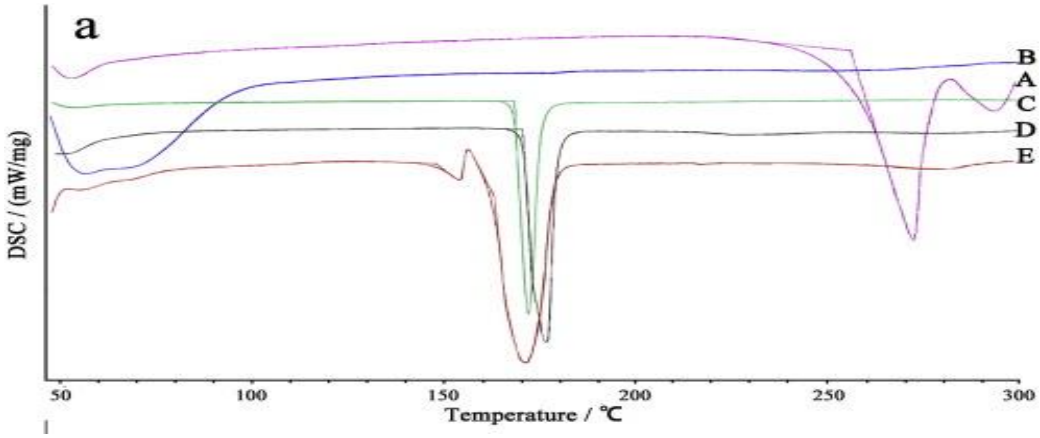
4. **Formulation trials using Microemulsion technique.**

   placebo trials were taken for microemulsion techniques using Liquid lipid, surfactants, co-surfactants and water. And after addition of drug to the same.

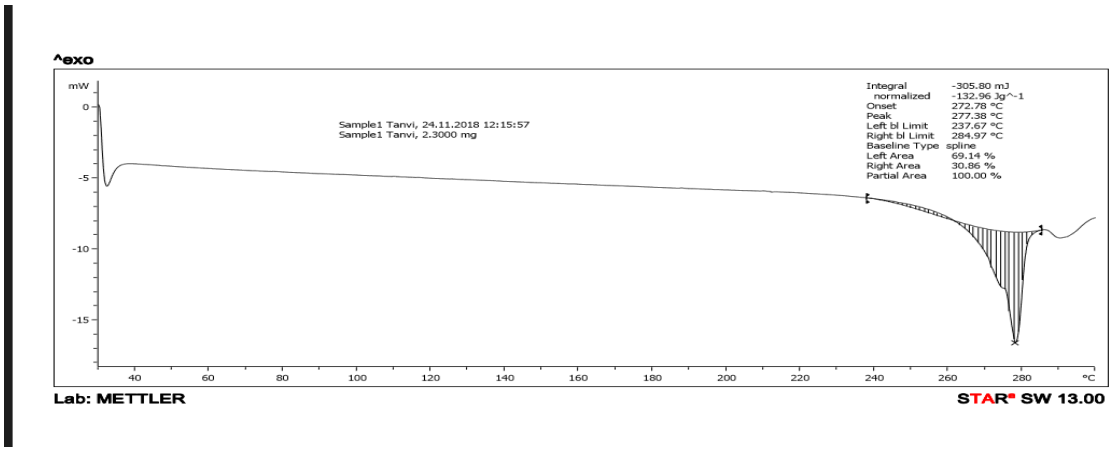
5. **RP-HPLC Analytical method development initiated using published method for Lurasidone hydrochloride.**

RESULTS :

Drug Excipient characterization: The Drug and excipient were characterized for Melting point by DSC. The DSC thermograph of the API was analysed for the degradation pattern of the drug and melting point as shown in Fig No 1. DSC Thermograph of drug shows onset at 258 ^{0} C and sharp peak proves that the drug is crystalline and it matches with the melting point of the drug. So we will select high shear hot homogenisation technique for NLC preparation



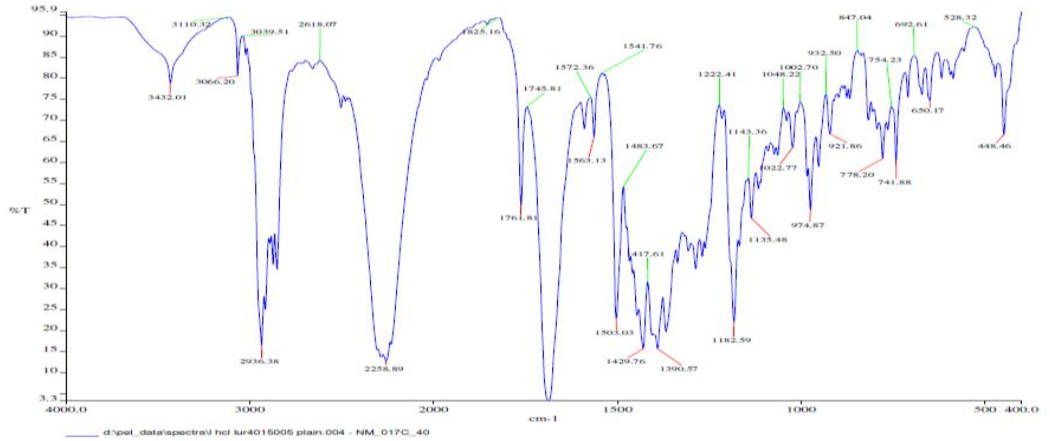
A



B

Figure 1 : DSC thermograph of Lurasidone hydrochloride A, Reference B Drug Sample

FTIR study:



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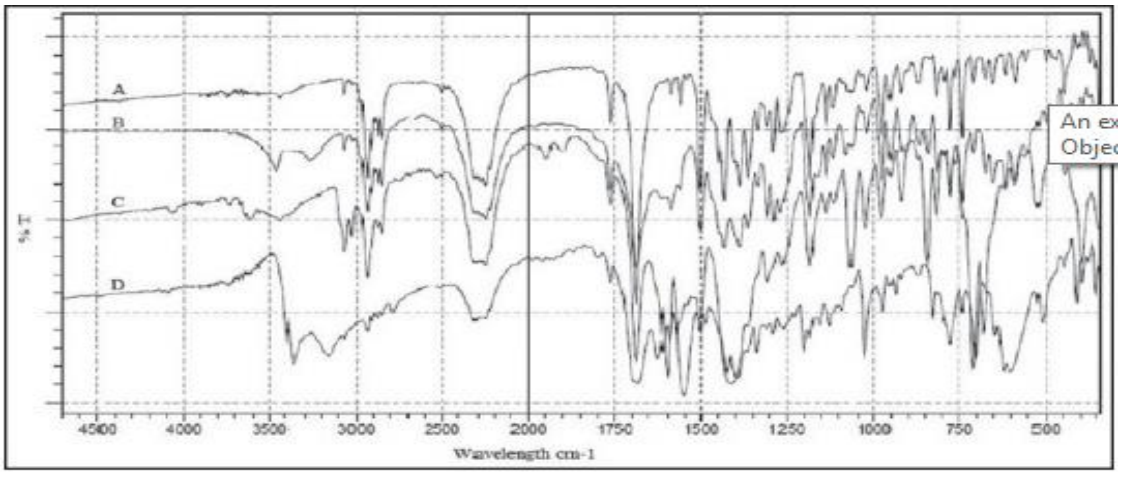


B

Figure 2 : IR Spectra of Lurasidone hydrochloroide A Drug Sample, B Reference

Lurasidone hydrochloride stretches	**Reference Peaks at Wavelengths**	**Observed Lurasidone HCL IR peaks at wavelengths**
Ar-H Stretch	2935	2936.4
C=O, Aryl Ketone	1686	1680
Ar C = C stretch	1503	1503
C-H bending	1400	1391
CN stretch	2259	2258.89
C-N stretch (ter. Amine)	1200	1183
C-Cl stretch	750	738

IR spectra of Lurasidone hydrochloride indicated Ar-H Stretch at wavelength 2936.4 nm which is matching with reference peak at wavelength 2935 reported in literature. Similarly

C=O, Aryl Ketone at 1680 nm corresponding to reference peak at wavelength 1686 reported in literature. Ar C = C stretch at 1503 nm corresponding to reference peak at wavelength 1503 reported in literature. C-H bending at 1391 nm corresponding to reference peak at wavelength 1400 reported in literature. CN stretch at 2258.89 nm corresponding to reference peak at wavelength 2259 reported in literature. C-N stretch (ter. Amine) stretch at 1183 nm corresponding to reference peak at wavelength 1200 reported in literature and C-Cl stretch stretch at 738 nm corresponding to reference peak at wavelength 750 reported in literature

Drug – Excipient studies: No change in Physical appearance observed from Initial and after 28 days by FTIR All peaks are within the reported range indicating purity of LRD. All the major peaks of LRD can also be seen in drug excipient physical mixture. Hence, there were no drug-excipients interactions [Figure 3 and 4].

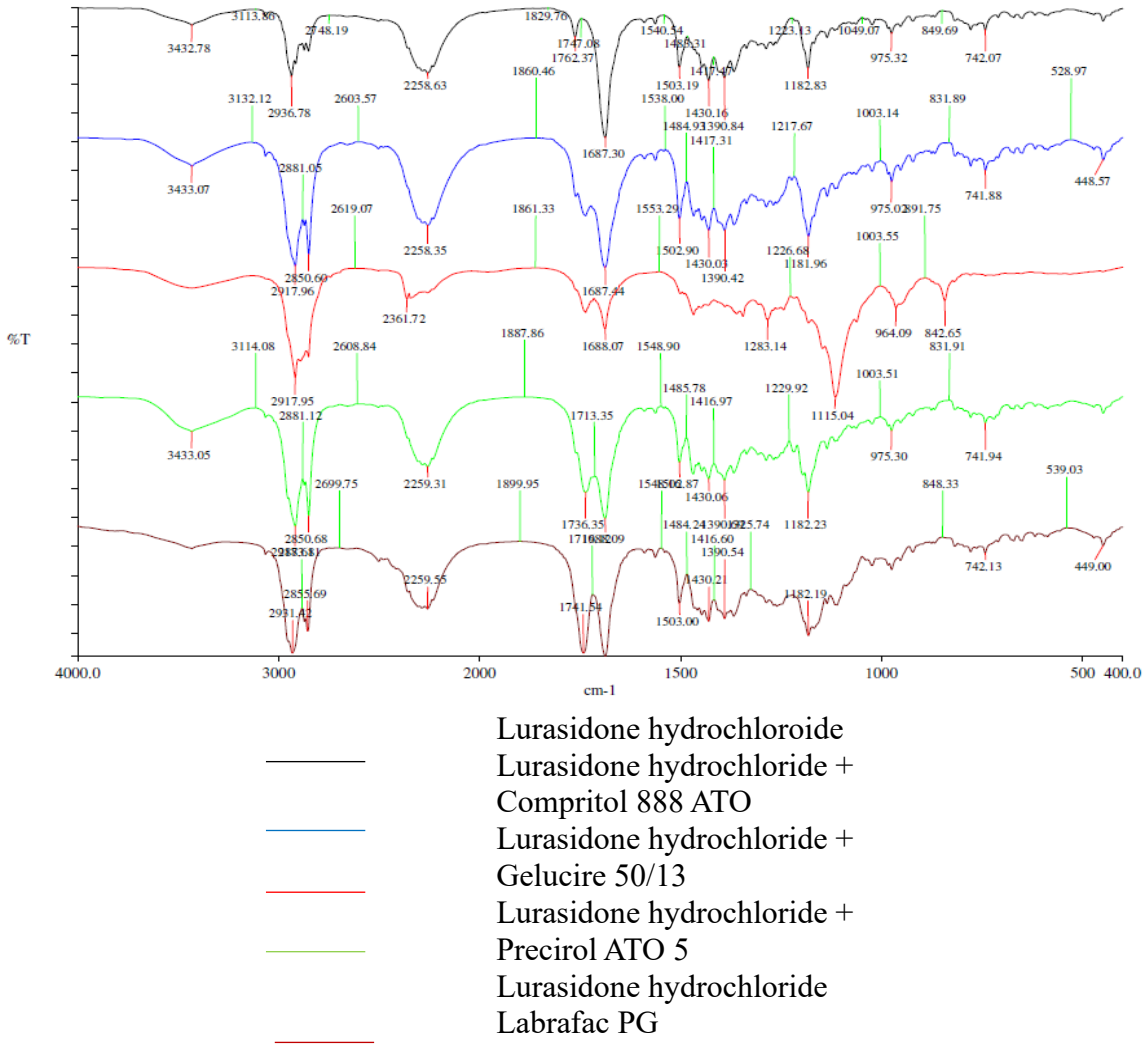


Figure 3 : IR Spectra of Lurasidone hydrochloride plain and with excipients at 0 days

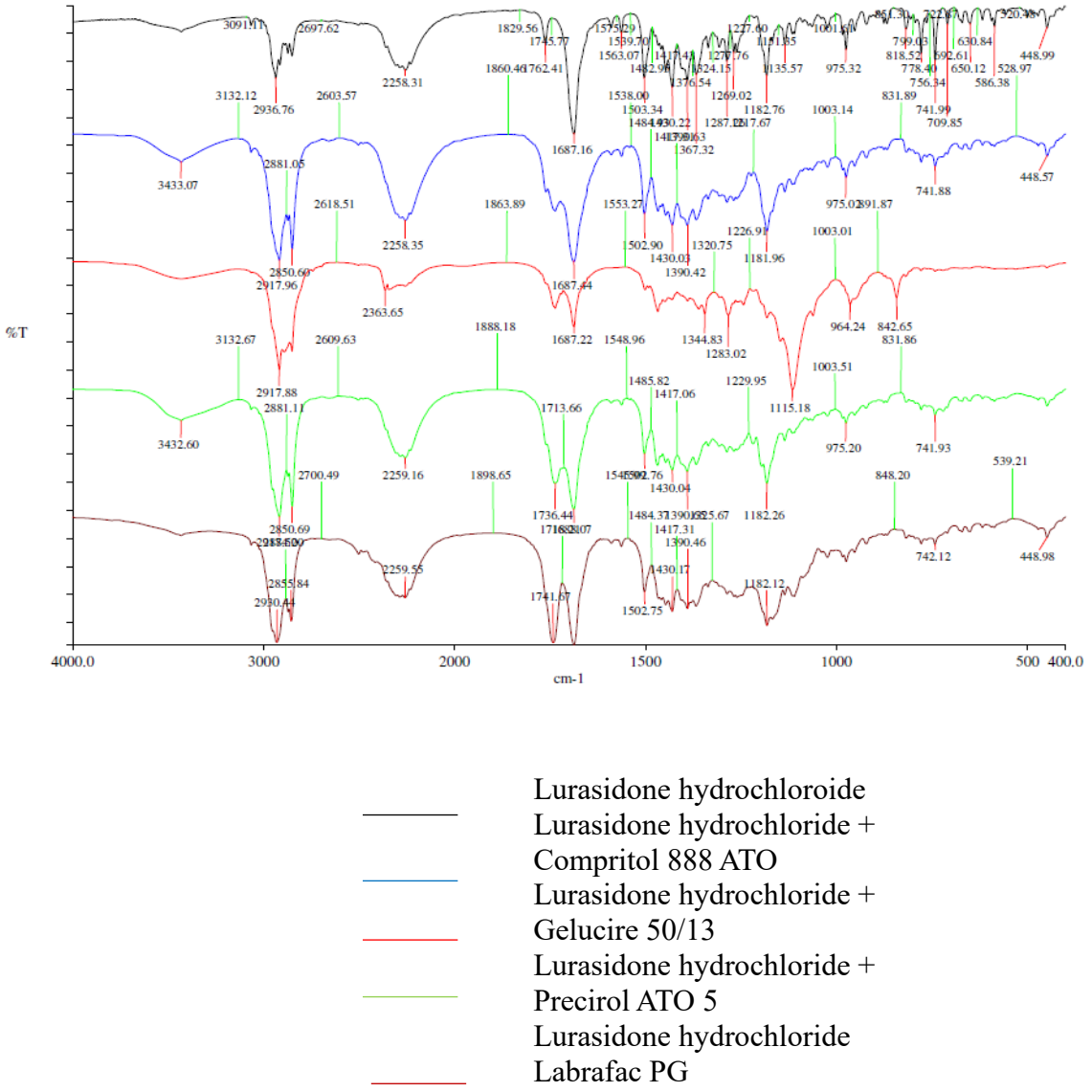


Figure 4 : IR Spectra of Lurasidone hydrochloride plain and with excipients at 28 days

Calibration curve in Mcllvaine buffer (Citrate Phosphate buffer) pH 3.5 : The calibration curve revealed correlation coefficient of 0.9998 as shown in Fig No 5 in the range of 10-80 ppm concentration as shown in Table no 1.

Table No 1: Concentration range for standard calibration plot

Conc. of drug in ppm	Absorbance at 315 nm
10	0.107
20	0.195
40	0.389
60	0.576
80	0.761

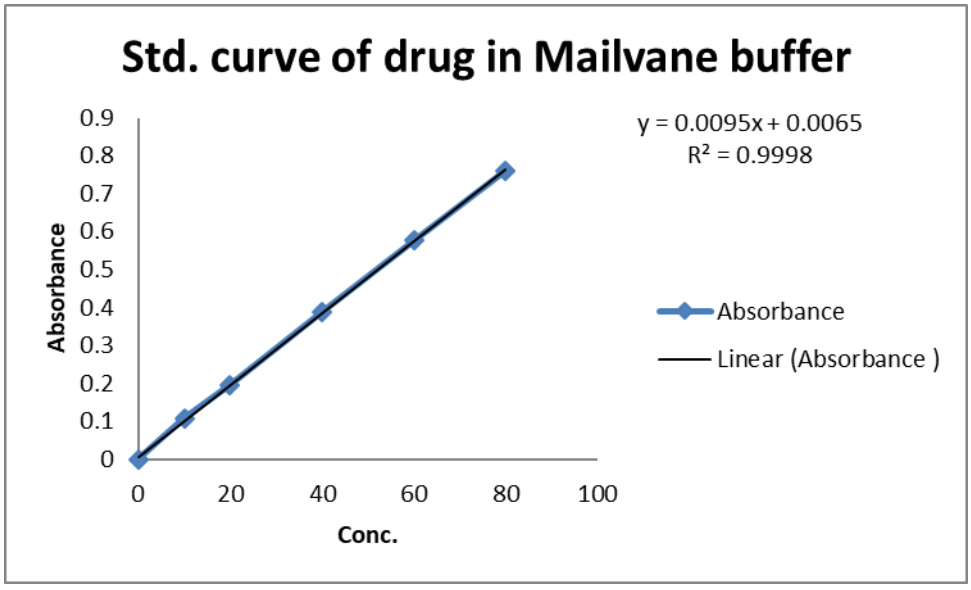


Fig No 6: Standard plot for Lurasidone HCL in Mcllvaine buffer (Citrate Phosphate buffer) pH 3.5

Calibration curve in Methanol: The calibration curve revealed correlation coefficient of 0.9991 as shown in Fig No 1 in the range of 5-25 ppm concentration as shown in Table no 2.

Table No 2: Concentration range for standard calibration plot

Conc. of drug in ppm	Absorbance at 234 nm
5	0.183
10	0.366
15	0.561
20	0.747
25	0.902

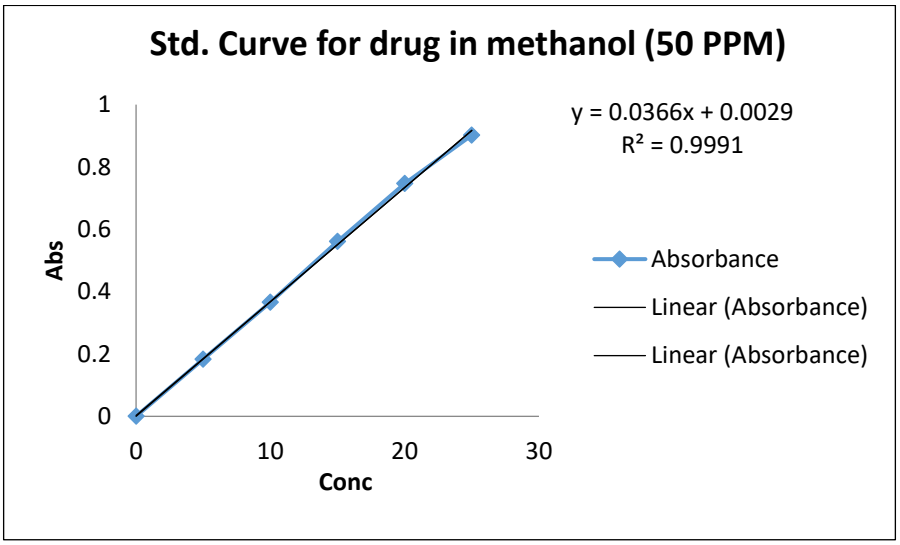


Fig No 6: Standard plot for Lurasidone HCL in MeOH

SOLUBILITY STUDIES: Solubility studies shows maximum solubility of drug in Glyceryl monostearate solid lipid as shown in Table 3 . From the observation of liquid lipid solubility, it was found that the solubility of drug is more in caproyl 90, cremophor RH40,Tween 80, solutol HP, Trioleate, oleic acid, PEG 400 as shown in Table 4 and Fig 7.

So we will initiate NLC preparation with these lipids and using Tween 80 and span 80 as surfactants. However depending on Entrapment Efficiency and particle size of NLC, the lipids and surfactants may vary.

Table 3: The solubility of API in Different Solid lipids

	Excipients	Solubility of drug (mg/1 g of lipid)
Solid lipids	Compritol 888 ATO	72
	Precirol ATO 5	60
	Glyceryl Monostearate	80
	Gelucire 50/13	74
	Cetyl Palmitate	53

The solubility of API in different liquid lipids, surfactants and co-surfactants in Phosphate buffer pH 5.6, 6.5 and 7.4

Solubility and calibration curve of drug was determined in Phosphate buffer pH 5.5, pH 6.5 and 7.4 and solubility of liquid lipids, surfactants and co-surfactants and in combination of liquid lipids was determined in each of these buffers.

PREPARATION OF PHOSPHATE BUFFER:

1. Potassium dihydrogen phosphate 0.2M: Dissolved 13.608gm of potassium dihydrogen phosphate in water and diluted with water up to 500 ml.
2. Sodium hydroxide ,0.2M: Dissolved 4g sodium hydroxide in water to produce a 40-60 % w/v solution and allow to stand.

For phosphate buffer preparation pH 5.5:

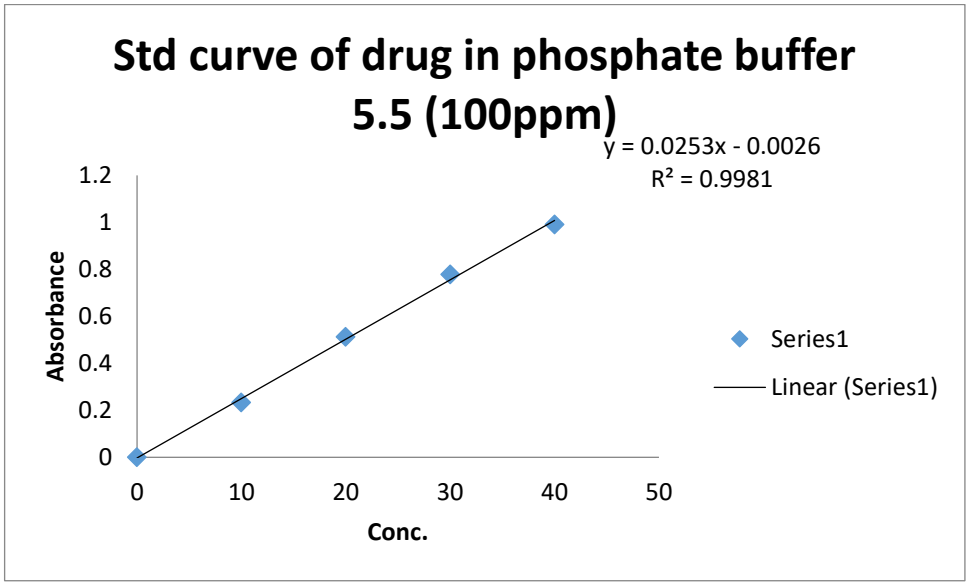
Placed 62.50ml of 0.2m potassium dihydrogen phosphate in a 250ml volumetric flask, added 4.26ml NaOH.

Standard curve in phosphate buffer pH 5.5:

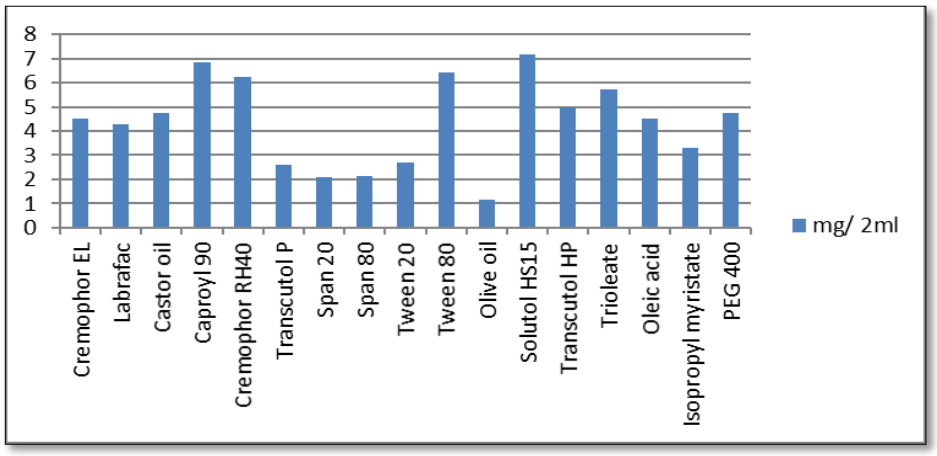
Dissolved 10 mg of drug in 2ml of methanol, then volume made up to 100ml with phosphate buffer pH5.5, checked absorbance at 234nm.

From stock solution withdraw 1,2,3,4 ml and makeup with phosphate buffer up to 10ml.

Conc.	Absorbance
10	0.233
20	0.512
30	0.778
40	0.991



Solubility of lipids in Phosphate buffer pH 5.5 :



<u>For phosphate buffer preparation pH 6.5</u>:

Placed 62.50ml of 0.2m potassium dihydrogen phosphate in a 250 mL volumetric flask, add 14.72 mL of 0.2M NaOH.

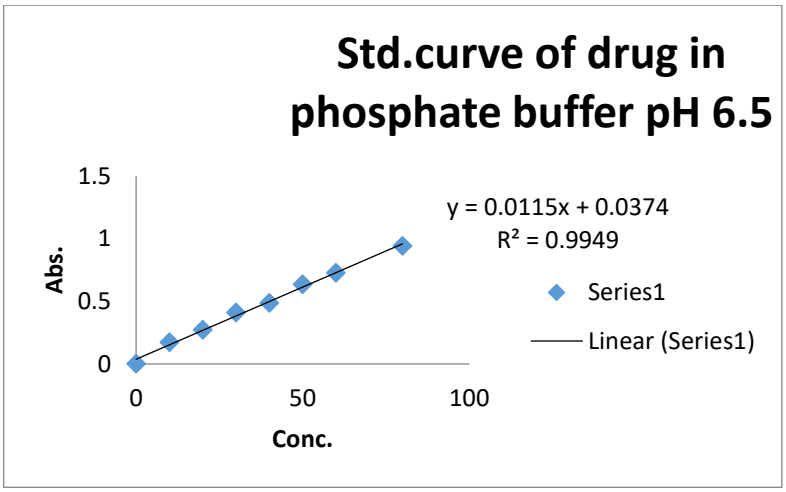
<u>Standard curve in phosphate buffer pH 6.5</u>:

Dissolved 10mg of drug in 2 mL of methanol, then volume makeup to 100ml with phosphate buffer pH 6.5, take a absorbance at 234 nm.

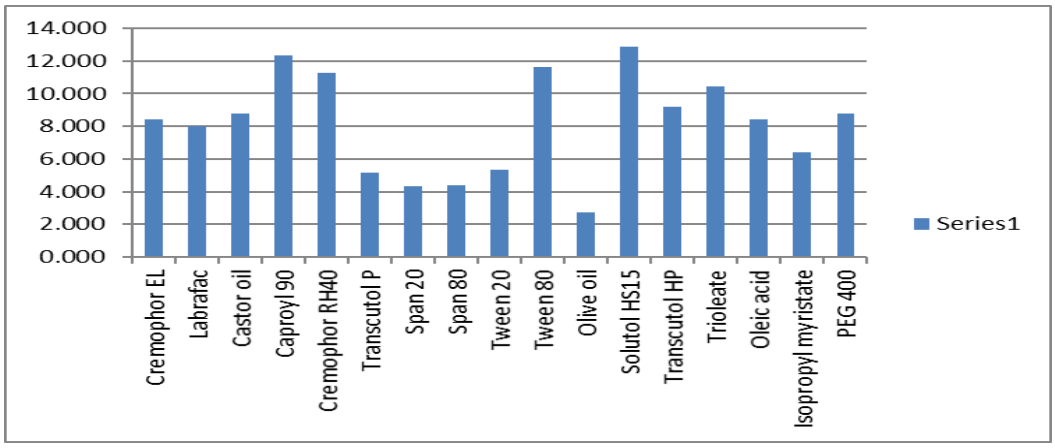
From stock solution withdrew 1,2,3,4,5,6,8 ml and makeup with phosphate buffer upto 10 mL.

Conc.	Absorbance
10	0.171
20	0.272
30	0.41
40	0.486
50	0.634

60	0.727
80	0.94



Solubility of lipid in Phosphate buffer pH 6.5



<u>For phosphate buffer preparation pH7.4</u>:

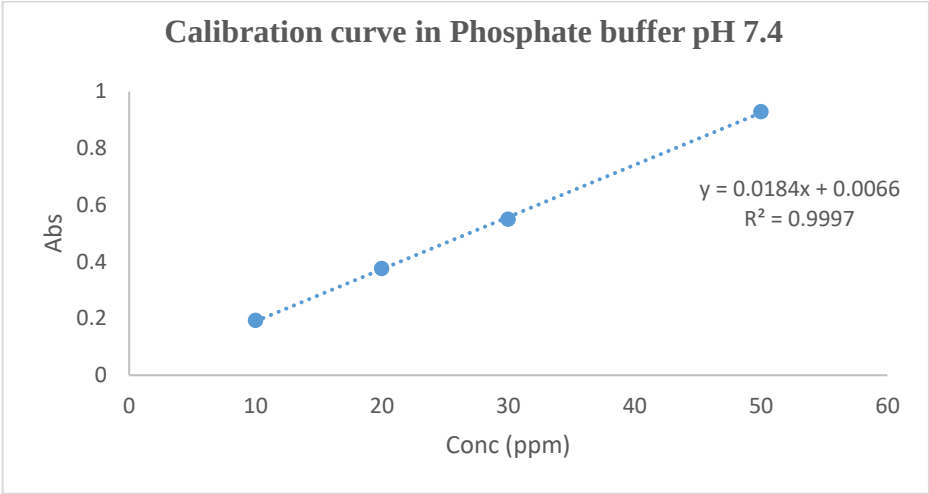
Place 62.50ml of 0.2 M potassium dihydrogen phosphate in a 250ml volumetric flask, add 139.1ml of 0.2M NaOH.

<u>Standard curve in phosphate buffer pH7.4</u>:

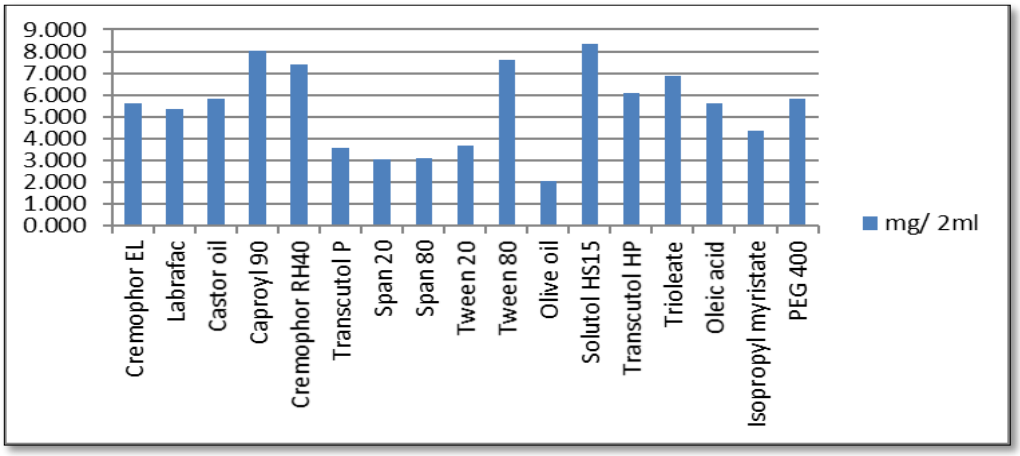
Dissolve 10mg of drug in 2ml of methanol, then volume makeup to 100ml with phosphate buffer pH7.4, take a absorbance at 234nm.

From stock solution withdraw 1,2,3,5 ml and makeup with phosphate buffer upto 10ml.

Conc.	Abs.
10	0.193
20	0.376
30	0.55
50	0.929



Solubility of lipids in Phosphate buffer pH 7.4:



Name of Lipid	Solubility (in mg/ 2ml) in Phosphate buffer pH 5.5	Solubility (in mg/ 2ml) in Phosphate buffer pH 6.5	Solubility (in mg/ 2ml) in Phosphate buffer pH 7.4
Cremophor EL	4.53	8.420	5.610
Labrafac	4.28	8.010	5.350
Castor oil	4.74	8.770	5.826
Caproyl 90	**6.85**	**12.340**	**8.042**
Cremophor RH40	**6.23**	**11.290**	**7.394**
Transcutol P	2.58	5.140	3.566
Span 20	2.11	4.340	3.069
Span 80	2.15	4.410	3.112
Tween 20	2.68	5.315	3.675
Tween 80	**6.44**	**11.645**	**7.610**
Olive oil	1.15	2.723	2.064

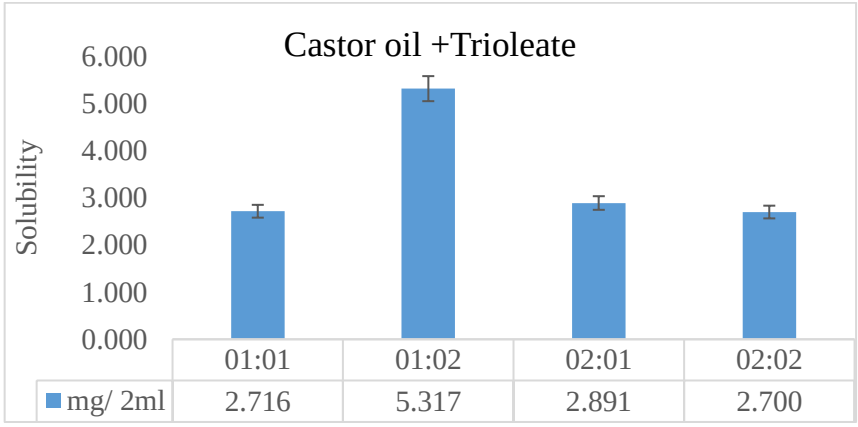
Solutol HS15	**7.15**	**12.845**	**8.356**
Transcutol HP	4.99	**9.210**	6.096
Trioleate	**5.72**	**10.445**	**6.864**
Oleic acid	4.53	8.428	5.610
Isopropyl myristate	3.32	6.393	4.345
PEG 400	4.74	8.776	5.826

Fig No 7: Solubility of drug in Liquid Lipids

Solubility in Combination lipids proposed for NLC preparation: The solubility of Lurasidone drug was checked in the combination of lipids to get the idea related to the entrapment of the drug.

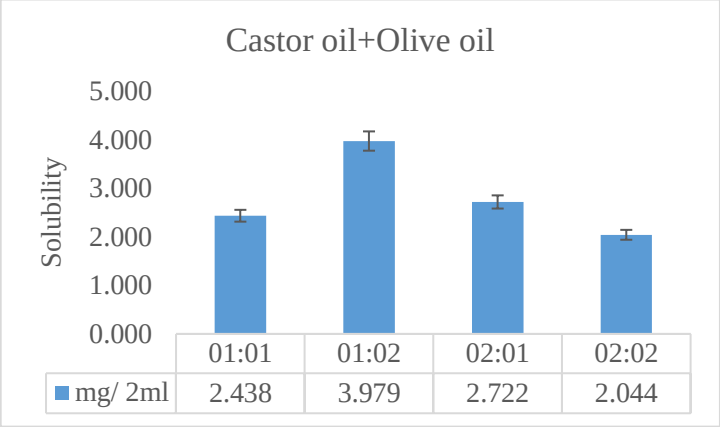
I. Lipid combination Castor oil +Trioleate

Castor oil +Trioleate (Ratio)	Concentration of drug(in mg/ 2ml)
01:01	2.716
01:02	5.317
02:01	2.891
02:02	2.700



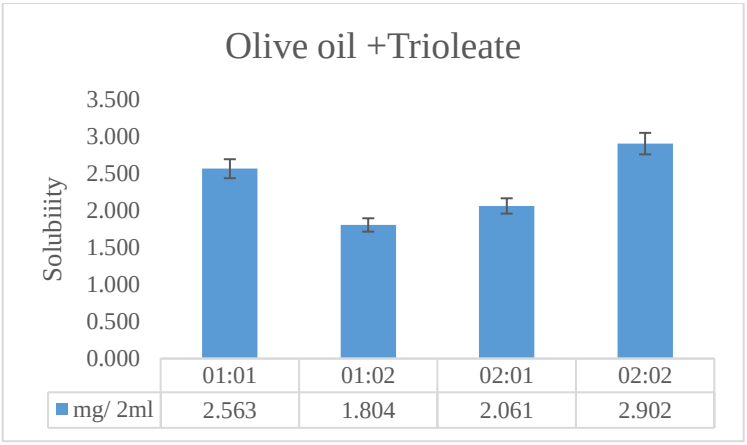
II. Lipid combination Castor oil +Olive oil

Castor oil+Olive oil (Ratio)	Concentration of drug(in mg/ 2ml)
01:01	2.438
01:02	3.979
02:01	2.722
02:02	2.044



III. ***Lipid combination : Olive oil + Trioleate***

Olive oil +Trioleate (Ratio)	**Concentration of drug (in mg/ 2ml)**
01:01	2.563
01:02	1.804
02:01	2.061
02:02	2.902



Calibration curve in Octanol: The calibration curve revealed correlation coefficient of 0.9977 as shown in Fig No 2 in the range of 5-40 ppm concentration as shown in Table no 5.

Table No 5: Concentration range for standard calibration plot

Concentration of drug in ppm	Absorbance at 234 nm
5	0.107
15	0.36
25	0.578
30	0.722
35	0.816
40	0.9

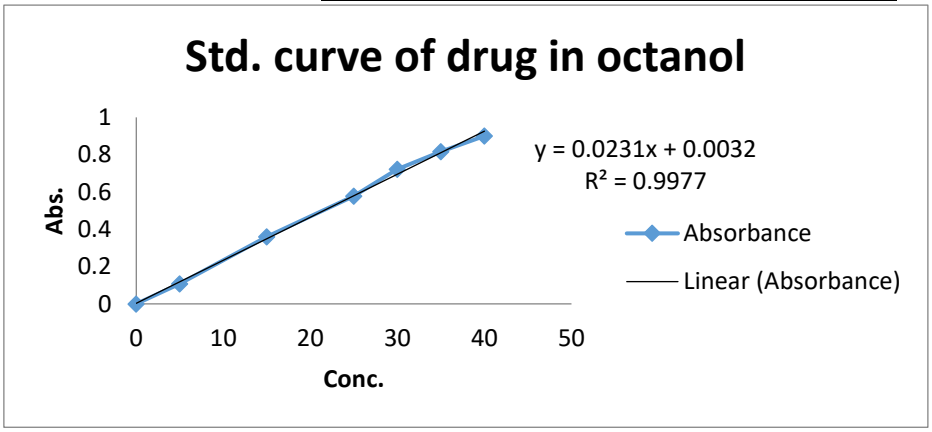


Fig No 8: Standard plot for Lurasidone HCL in Octanol

Table 6 : The solubility of API in water and Octanol

Samples	Absorbance at 234 nm A	Absorbance at 234 nm B	mean (y)	c	y-c	m	x= y-c/m	Dilution 1	Dilution 2	mg/ml
water	0.122	0.177	0.2105	0.0032	0.2073	0.0231	8.97403	89.74026	897.4026	0.8974
octanol	0.758	0.737	1.1265	0.0032	1.1233	0.0231	48.6277	486.2771	4862.771	4.86277

Formula for Log P value or Ko/w=$\frac{\text{Coil - Caq}}{\text{Caq}}$

Coil	Caq	Coil-Caq	Ko/w
4.863	0.897	3.966	4.4214

RESULT : Log p value of 4.4214 reveals that Lurasidone hydrochloride is lipophilic drug and iincreases chances of crossing blood brain barrier of NLC formulation.

3 Formulation of placebo NLC trials

Role	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14
Solid Lipid	GMS	11	11	11	6	6	6	-	-	-	-	-	-	-	-
Solid Lipid	Precirol	-	-	-	-	-	-	3	3	3	3	3	3	-	
Liquid Lipid	Caproyl 90	-	-	-	-	-	-		-	-	-	-	-	-	-
Surfactant	Tween 80	6	6	6	-	-	-	-	-	-	7	7	7	4	4
Liquid Lipid	Trioleate	3	-	-	8	-	-	10	-	-	10	-	-	8	
Surfactant	Solutol HS15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Solid Lipid	Compritol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Liquid Lipid	Oleic Acid	-	3	-	-	-	-	-	-	-	-	-	-	-	-
Liquid Lipid	olive oil	-	-		-	-	8	-	-	10	-	-	10	-	8
Liquid Lipid	Castor oil	-	-	3	-	8	-	-	10	-	-	10	-	8	8
Surfactant	Span 80	-	-	-	6	6	6	7	7	7	-	-	-	-	-
base	Water	80	80	80	80	80	80	80	80	80	80	80	80	80	80
	Batch size	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
	Time(in mins)	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	Temperature(˚C)	80-85	80-85	80-85	80-85	80-85	80-85	70-75	70-75	70-75	70-75	75-80	75-80	75-80	75-80
	RPM	1500	1500	1500	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

	HPH	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Probe sonicator		5 mins	5 mins	-	-	-	-	-	-	-	-	-	-	-
	Remark	**Phase separation**	**Phase separation**	**Phase separation**	**Solidify**	**Solidify**	**Phase separation**	**Phase separation**	**Solidify**	**Solidify**	**Solidify**	**Phase separation**	**Phase separation**	**Phase separation**	**Phase separation**

Role	**Ingredients**	**F15**	**F16**	**F17**	**F18**	**F19**	**F20**	**F21**	**F22**	**F23**	**F24**	**F25**	**F26**	**F27**	**F28**
Solid Lipid	GMS	-	-	4	4.5	-	4.8	2.4	4	-	-	-	5	-	-
Solid Lipid	Precirol	-	1	-	-	6	-	-	-	5	5	5	12	6	6
Liquid Lipid	Caproyl 90	-	-	-	-	8	-	-	5	8	-	-	-	9	9
Surfactant	Tween 80	4	6	5	-	8	10	11	9	10	10	10	9	9	9
Liquid Lipid	Trioleate	8	10	-	-	-	-	-	-	-	-	-	-	-	-
Surfactant	Solutol HS15	-	-	5	4	-	-	-	-	-	-	-	-	-	-
Solid Lipid	Compritol		1	4	4.5	-	-	-	-	-	-	-	-	-	-
Liquid Lipid	Oleic Acid	-	-	7	7	-	-	-	-	-	-	-	-	-	-
Liquid Lipid	olive oil	8	-	-	-	-	8	-	-	-	8	-	-	-	-

Liquid Lipid	Castor oil	-	-	-	-	-	-	8	-	-	-	8	-	-	-
Surfactant	Span 80	-	-	-	-	-	-	-	-	-	-	-	-	-	-
base	Water	80	82	75	80	78	77.2	78.6	82	77	77	77	74	76	76
	Batch size	5 ml	5 ml	5 ml	5 ml	5 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml
	Drug in mg	-	-	-	-	-	400	400	400	400	400	400	700	550	700
	Time (min)	20	30	60	60	60	60	60	60	60	60	60	60	60	60
	Temperature (˚C)	75-80	75-81	80-85	80-85	80-85	80-85	80-85	80-85	80-85	85-90	80-85	80-85	80-85	85-90
	RPM	2000	1500	1900	1900	2000	2000	1700	2000	2000	1920	2000	2000	2000	2000
	HPH						5000psi for 10 cycles	4000psi for 15 cycles							
	Probe sonicate						16 mins	16 mins							
	Remark	**Phase Separation**	**Solidify**	**Solidify**	**Solidify**	**Phase Separation**	**Turbid solution layers separated**	**Turbid solution layers separated**	**Liquid stable**	**Liquid stable**	**Phase separation**	**Phase separation**	**Stable but thick**	**Stable but thick**	**Stable but thick**

Hot emulsification technique followed by probe sonication was selected for formulating Lurasidone hydrochloride NLC.

Maximum solubility of drug was observed in Methanol with λmax of 230nm after UV scan between 200-400nm. Solubility studies of drug showed, maximum solubility of Lurasidone hydrochloride in wide range of solid lipids, liquid lipids and surfactants.

After several formulation trials, NLC using Glyceryl monostearate as solid lipid, combination of Precirol and Caproyl-90 as liquid lipids and Tween 80 as surfactant were selected as NLC ingredients. Hot emulsification technique followed by probe sonication gave stable NLC preparation which was stable at accelerated stability condition of 40ºC/75% RH for 2 months. This formulation was taken forward for optimization using Design of experiment. (DOE)

2. Design of Experiment (DoE)

Design Expert® v10 (DX10), Stat -Ease-Inc Minneapolis, USA software was used to illustrate the response surface model by obtaining the combination of values. A three -factor three level Box Behnken experimental Design (BBD) was used to optimise the procedure.

A design consisted of three parts, each of two fully levelled factors and a third factor set at zero level. Design expert software was used for investigating the quadratic response surfaces, which were represented by second order polynomial model.

From preliminary experiment, the independent variables selected were % of Solid lipid, % of Surfactants and sonication time; each of these were selected at their low, medium and high levels. The dependent variables were particle size (nm) and entrapment efficiency (%).

File Version	11.1.0.1		
Study Type	Response Surface	**Subtype**	Randomized
Design Type	Box-Behnken	**Runs**	15
Design Model	Quadratic	**Blocks**	No Blocks
Build Time (ms)	1.0000		

The coded and actual values of the dependent and independent variables of BBD are as shown in below table. Each factor is studied and coded at three basic levels.

Factors (Independent variables)	**Levels used, actual (coded values with 3 levels)**		
	Low (-1)	**Medium (0)**	**High (+1)**
A: Solid Lipid (%)	50	55	60
B: Surfactant (%)	2	3	4
C: Sonication time (minutes)	10	15	20
Dependent variables selected are R1: Particle size (nm) R2: Entrapment efficiency (%)			

The three factors needed in BBD design gave 12 runs plus three replicates at the centre point (Total 15 runs)

Run	Coded values A: Solid lipid (%)	Coded values B: Surfactant (%)	Coded values C: Sonication time (minutes)	Response 1 (R1): Particle size (nm)	Response 2 (R2): Entrapment efficiency (%)
1	-1	0	-1	53.08	92.97
2	0	1	-1	102.7	95.77
3	0	-1	1	159.5	92.73
4	1	0	-1	162.99	96.33
5	1	-1	0	139.1	94.8
6	1	1	0	31.04	94.08
7	-1	1	0	41.71	88.27
8	0	1	1	90.7	87.24
9	1	0	1	46.43	93.8
10	-1	0	1	31.55	87.94
11	0	0	0	55.02	89.89
12	0	0	0	55.02	90.7
13	0	-1	-1	168.38	92.47
14	0	0	0	55.02	90.64
15	-1	-1	0	77.89	88.22

The significance of the model and model terms that were generated were analysed using analysis of variance (ANOVA). The ANOVA for quadratic model for response 1 for particle size was significant.

Particle size (R1) =55+21.94A-34.84B-19.89C-17.97AB-23.71AC-0.78BC-19.72A^2+37.14B^2+38.16C^2

The ANOVA for quadratic model for response 2 for EE was significant.

EE (R2) =+90+ 2.70A-0.36B-1.98C-0.193AB+0.63AC-2.19BC+0.82A^2+ 0.113B^2+1.53^2

100 solutions found for each of response i.e Particle size and EE. 1 solution in each with desirability 1 were selected for optimising the batches further.

Entrapment efficiency

100 solutions found for response 2 i.e % EE. 1 solution as below with desirability 0.952 was selected for optimizing the batch further.

Run no. 7 with parameters, Solid lipid 0 (50%), Surfactant mix 0 (3%) and sonication time 0 (15mins) were selected for further characterization.

3. RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF LURASIDONE HYDROCHLORIDE IN BULK

There are several UV spectroscopy[32] and HPLC[33-35] methods reported in literature for estimation of Lurasidone hydrochloride in bulk and tablet dosage forms. One of the reported RP-HPLC method[36] was run which was optimized and validated further as per ICH guidelines to give more cost effective, precise and accurate method for the estimation of Lurasidone hydrochloride in bulk. Estimation of Lurasidone hydrochloride in NLC formulation using the validated method is in-progress.

Differences between reported[36] and developed method, is as shown in table below

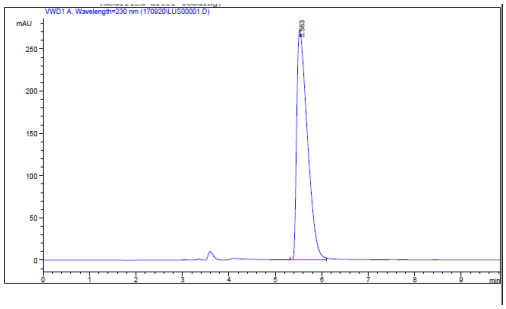
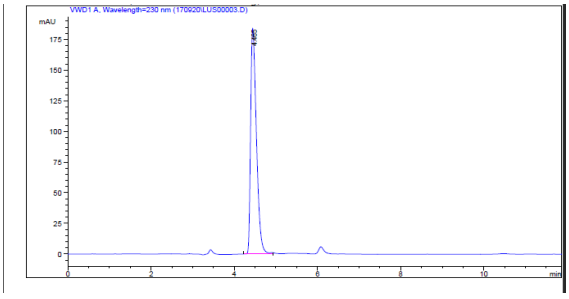
Parameters	**Reported method**	**Developed method**	**Observations Developed method vs reported method**
HPLC column	Eclipse $C_{18,}$ Column (4.6 x 100 mm)	Fortis $C_{18,}$ Column (4.6 x 100 mm)	
Particle size	3.5 μm	2.5 μm	Finer particle size gave better resolution
Optimized mobile phase	Methanol and water containing 0.01% Ortho Phosphoric Acid in 70:30 (v/v) ratio	Methanol and water containing 0. 1% Ortho Phosphoric Acid in 60:40 (v/v) ratio	Lower concentration of methanol and hence more cost-effective method
flow rate	1 ml/min	0.7 ml/min	Lower flow rate

Sample volume	20 µl	20 µl	
HPLC system		Agilent Tech. Gradient System with Auto injector using UV (DAD) G13148 detector	
Detection wavelength	240nm	230 nm	

Results comparison between reported method and validated method is in table below

Parameters	**Reported method**	**Developed method**	**Observations Developed method vs reported method**
Retention time	2.24 minutes	4.30 minutes	
Linearity in the concentration range	2.5-15 µg/ml	10-50 µg/ml	Linearity observed in wider concentration range
Correlation coefficient value r^2	0.999	0.999	
Limit of detection (LOD)	0.524 µg/ml	0.091 µg/ml	The developed method has lower LOD
Limit of quantification (LOQ)	1.58 µg/ml	0.275 µg/ml	The developed method has lower LOQ
Accuracy studies levels	50%,100% and 150%	80%,100% and 120%	The developed method is accurate at narrow range of levels
Percentage recovery range	100.1% to 100.6%	100.2% to 100.9%	

Interday and Intraday precision studies relative concentration ranges and %RSD	5,10,15 µg/ml <2%	10,30 50 µg/ml <1%	The developed method is precise over wide concentration range with lower RSD
Robustness of method with relative standard deviation (RSD)		<2%	
Change in wavelength by	2nm	1nm	
Change in Mobile phase concentration	Not done	Done	
Change in flow rate by	0.2 ml	0.1ml	
The drug was checked for degradation in acid, alkali, neutral and photolytic forced degradation conditions	Studies not done	Checked after 1hour, 2 hours, 6 hours	
Oxidative forced degradation condition	Studies not done	Checked after 1 hour, 2 hours, 6 hours and 24 hours	

TRIAL-01 70 MEOH+ 30 0.1 % OPA)-0.7 ML (250 X 4.6 MM)50 MCG 	TRIAL-02 (75 MEOH+ 25 0.1 % OPA)-0.7 ML (250 X 4.6 MM)50 MCG 

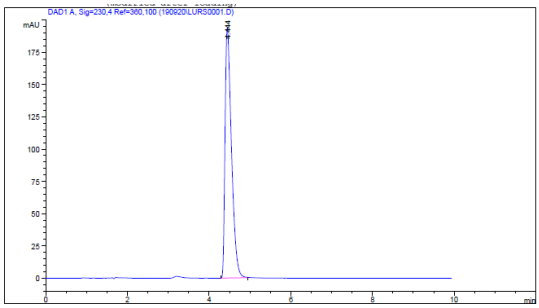
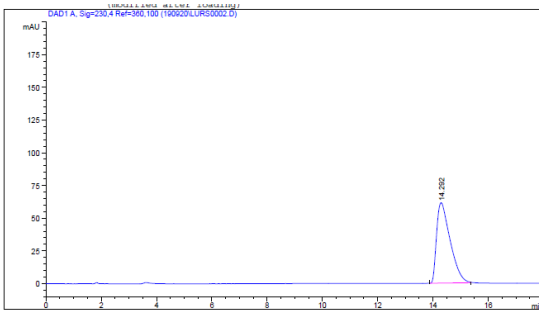
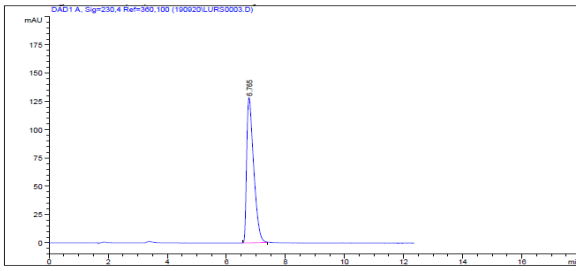
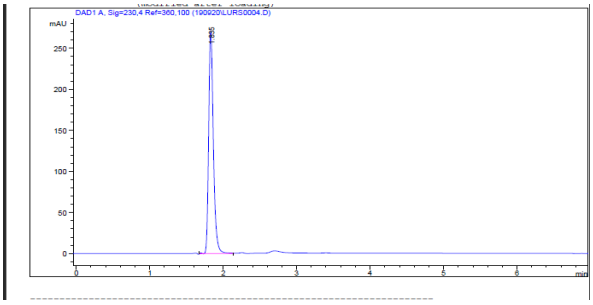
TRIAL-03 (60 MEOH+ 40 (0.1 % OPA)-0.7 ML-(230 NM COLUMN 100 X 4.6 mm x 2.5 micron) 	TRIAL-04 (50 MEOH+ 50 (0.1 % OPA)-0.7 ML-230 NM COLUMN = 100 X 4.6 mm x 2.5 micron) 
TRIAL-05 (55 MEOH+ 45 (0.1 % OPA)-0.7 ML-230 NM COLUMN = 100 X 4.6 mm x 2.5 micron) 	TRIAL-06 (75 MEOH+ 25 (0.1 % OPA)-0.7 ML-230 NM COLUMN = 100 X 4.6 mm x 2.5 micron) 

Figure 2: Chromatograms of different mobile phases trials and columns width and particle size packings.

The chromatographic conditions from the optimisation studies using various set parameters were selected. Retention time of Lurasidone hydrochloride was obtained at 4.444 ± 0.02 mins with mobile phase consisting of Methanol + buffer containing 0.1% Orthophosphoric acid in water in ratio of 60:40 (%v/v). The column selected was 100 x 4.6 mm with particle size packing 2.5µ. The detection wavelength was set to 230 nm with a flow rate of 0.7ml/min.

METHOD VALIDATION

The optimized method was validated [8,9] as per ICH guidelines [10]

System Suitability

Specificity

Specificity of the method was done by comparing the chromatograph of Lurasidone hydrochloride with blank chromatograph. Figure 3 shows Chromatograph of Lurasidone hydrochloride and blank

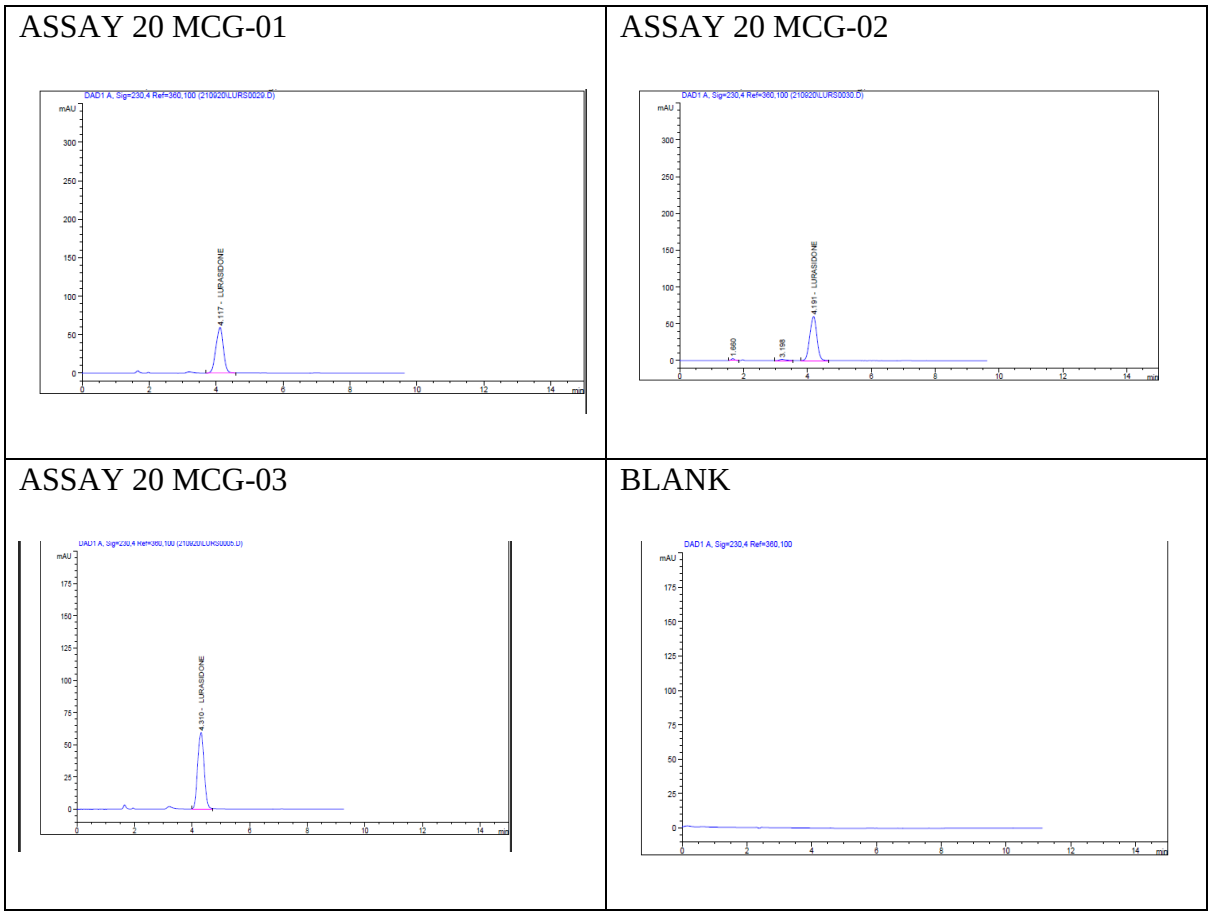


Figure 3 Chromatograph of Lurasidone hydrochloride and blank

Accuracy

The accuracy of the analytical method determines how close the test results are with actual values. Accuracy was done by recovery method. In this percent recovery of the analyte was determined. The recovery of standard solution of Lurasidone hydrochloride (10µg/ml) was estimated at 3 different levels (80%, 100% and 120%). Each level was recovered in 3 consecutive series. Percent recovery in Table 1 shows that method is accurate.

Table 1: Percent recovery of Lurasidone hydrochloride

Sample No.	% spike level	Amount of Lurasidone hydrochloride spiked (µg/ml)	Amount of Lurasidone hydrochloride recovered (µg/ml)	% recovery	Mean % recovery
1	80	8	8.0512	100.6406	100.18
2		8	7.9849	99.8123	
3		8	8.0069	100.0866	
1	100	10	10.0918	100.9185	100.79
2		10	10.0522	100.5229	
3		10	10.0915	100.9152	
1	120	12	12.0516	100.4307	100.86
2		12	12.1446	101.2057	
3		12	12.1116	100.9303	

Precision

The precision of an analytical method determines the closeness of a series of individual analyte measurements applied repeatedly to multiple aliquots of the same sample. It is calculated as a Relative Standard Deviation (RSD). The intraday (same day) precision study was carried out by estimating the area peak responses of Lurasidone hydrochloride standard solution for 10, 30 and 50µg/ml. Each concentration was repeated three times. So total 9 estimations on same day. The interday (2 different days) precision study was carried out by estimating the area peak responses of Lurasidone hydrochloride standard solution for 10, 30 and 50µg/ml. Each concentration was repeated three times. So total 9 estimations on next day. Intraday and interday results in Table 2 depicts that method is precise.

Table 2: Intraday and Interday precision for Lurasidone hydrochloride

Sample No.	Conc. µg/ml	Peak area of Lurasidone hydrochloride Interday	Mean	%RSD	Peak area of Lurasidone hydrochloride Intraday	Mean	%RSD
1	10	473.308	473.585	0.89	479.2342		0.890
2		473.631			476.4116		
3		473.815			475.4886	477.045	
1	30	1403.303	1397.041	0.3502	1407.4932		0.113
2		1396.46			1403.822		
3		1391.36			1404.5721	1405.296	

1	50	2302.551	2300.083	0.0768	2298.642		0.087
2		2298.516			2302.902		
3		2299.182			2302.840	2301.461	

Repeatability

Repeatability was determined by injecting 50 µg/ml three times. Corresponding peak areas of the chromatograms generated were measured and %RSD calculated. The method is precise if %RSD less than 2%. Table 3 shows system repeatability results. Since %RSD is 0.0417, the method is precise.

Table 3: Repeatability results

Sr No.	Conc. µg/ml	Area I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	50	2298.4196	2303.0842	2298.3703	2299.96	49.5200	99.0455	0.9600	0.0417

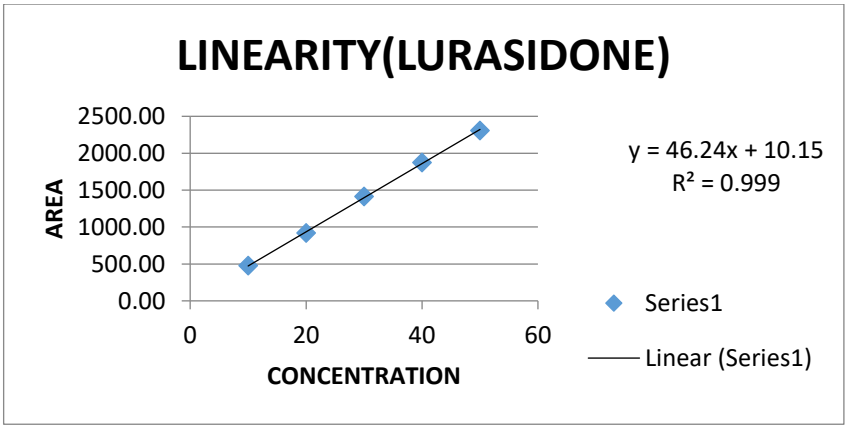
Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. Linearity of Lurasidone hydrochloride was studied for 10, 20, 30, 40 and 50 µg/ml concentrations. These solutions were run in HPLC system for 10 mins at flow rate of 0.7ml/min. The Chromatographs shows concentration of Lurasidone hydrochloride on x axis and peak area on y axis. The peak area response vs concentration prepared was used for concentration of calibration curve. The calibration curve was found to be accurately fit with very high correlation of 0.999. The regression equation was found to be y = 46.24x +10.15 (Fig 5). The linearity study results were given in Table 4. Figure 6 shows linearity chromatographs of Lurasidone hydrochloride.

Table 4: Linearity study results of Lurasidone hydrochloride

Sr No.	**Conc**	**Area I**	**Area II**	**Area III**	**Mean Area**	**SD**	**%RSD**
1	10	475.3453	474.7887	475.9544	475.07	0.58	0.12
2	20	918.3244	917.7009	915.4232	917.15	1.53	0.17
3	30	1412.1646	1412.2108	1408.4041	1412.19	2.18	0.15
4	40	1873.1696	1874.0678	1874.1826	1873.81	0.55	0.03
5	50	2307.5402	2308.458	2310.4863	2308.83	1.51	0.07
					Avg. SD	**1.27**	

Figure 6: Linear calibration curve of Lurasidone hydrochloride



Limit of detection (LOD) and Limit of quantification (LOQ)

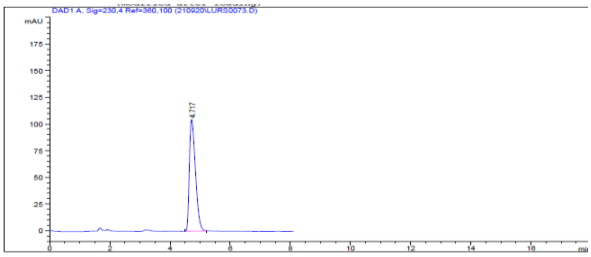
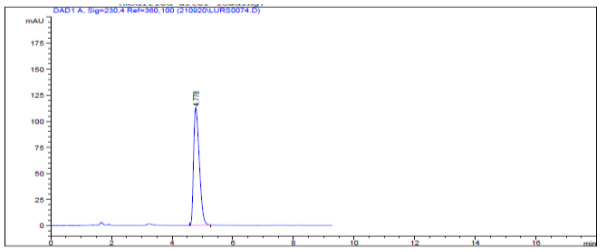
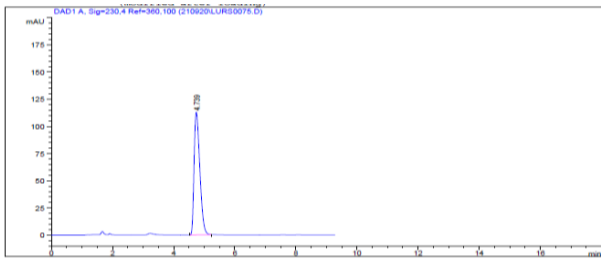
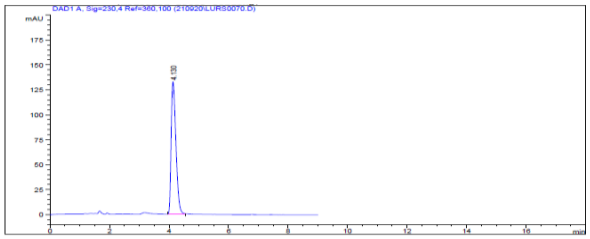
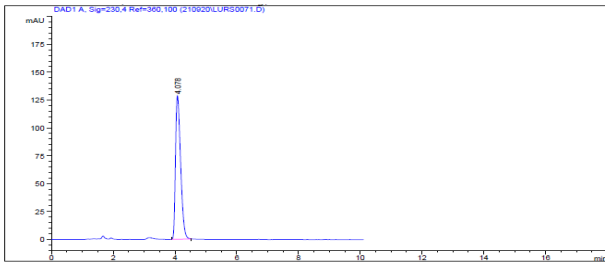
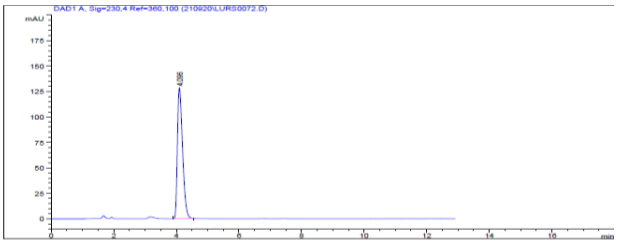
The LOD and LOQ values determines the sensitivity of the proposed method. LOD and LOQ are calculated from Standard deviations and slope determined by calibration curve in Linearity.

Robustness

Robustness of method is determined by applying deliberate small changes in analysis condition. To check there is no change in retention time and chromatographic conditions.

Robustness of the proposed method was done by variation of wavelength by 1nm, flow rate by 0.1min/ml and mobile phase composition, each test was repeated at 3 levels. The RSD was less than 2% in each of these conditions indicating that method is robust. Figure7 representative Chromatographs with change in mobile phase, flow rate and wavelength respectively

Figure 7: Chromatographs with change in mobile phase

ROUBUSTNESS COMP CHANGES 59 MEOH+ 41 (0.1 % OPA)0.7 ML -01(30 MCG) 	ROUBUSTNESS COMP CHANGES 59 MEOH+ 41 (0.1 % OPA)0.7 ML -02(30 MCG) 
ROUBUSTNESS COMP CHANGES 59 MEOH+ 41 (0.1 % OPA)0.7 ML-03(30 MCG) 	ROUBUSTNESS COMP CHANGES 61 MEOH+ 39 (0.1 % OPA)0.7 ML -01(30 MCG) 
ROUBUSTNESS COMP CHANGES 61 MEOH+ 39 (0.1 % OPA)0.7 ML -02(30 MCG 	ROUBUSTNESS COMP CHANGES 61 MEOH+ 39 (0.1 % OPA)0.7 ML -03(30 MCG) 

FORCED DEGRADATION STUDIES

Acid degradation studies in 0.1N hydrochloric acid.

To 0.3 ml (30 mcg) of Lurasidone hydrochloride stock solution, added 5 ml of 0.1N HCL and made up the volume with mobile phase. After 1 hour, 2 hours and 6 hours HPLC readings were taken. Table 5 shows acid degradation results

Table 5: Acid degradation of Lurasidone hydrochloride

No. of hours	Area of Standard	Area of degraded Sample	Degraded upto %	Actual % degradation
1	1412.19	1358.6298	96.21	3.79
2	1412.19	1351.0498	95.67	4.33
6	1412.19	1292.783	91.54	8.46

Alkali degradation studies in 0.1N sodium hydroxide solution.

To 0.3 ml (30 mcg) of Lurasidone hydrochloride stock solution, added 5 ml of 0.1N NaOH solution and made up the volume with mobile phase. After 1 hour, 2 hours and 6 hours HPLC readings were taken. Table 6 shows acid degradation results

Table 6: Alkali degradation of Lurasidone hydrochloride

No. of hours	**Area of Standard**	**Area of degraded Sample**	**Degraded upto %**	**Actual % degradation**
1	1412.19	1298.0734	91.92	8.08
2	1412.19	1277.6695	90.47	9.53
6	1412.19	1229.4122	87.06	12.94

Neutral degradation studies in water.

To 0.3 ml (30 mcg) of Lurasidone hydrochloride stock solution, added 5 ml of water and made up the volume with mobile phase. After 1 hour, 2 hours and 6 hours HPLC readings were taken. Table 7 shows acid degradation results

Table 7: Neutral degradation of Lurasidone hydrochloride

No. of hours	Area of Standard	Area of degraded Sample	Degraded upto %	Actual % degradation
1	1412.19	1353.25	95.83	4.17
2	1412.19	1412.19	1347.39	95.41
6	1412.19	1412.19	1340.1041	94.90

Peroxide degradation studies in Hydrogen peroxide (H_2O_2) solution.

To 0.3 ml (30 mcg) of Lurasidone hydrochloride stock solution, added 5 ml of H_2O_2 solution and made up the volume with mobile phase. After 1 hour, 2 hours and 6 hours HPLC readings were taken. Table 8 shows oxidative degradation results and figure 13 shows chromatograms.

Table 8: Peroxide degradation of Lurasidone hydrochloride

No. of hours	**Area of Standard**	**Area of degraded Sample**	**Degraded upto %**	**Actual % degradation**
1	1412.19	1406.5274	99.60	0.40
2	1412.19	1402.44	99.31	0.69
6	1412.19	1392.7793	98.63	1.37
24	1412.19	1346.9736	95.38	4.62

Photolytic degradation

Photodegraded Lurasidone hydrochloride 30 MCG sample was injected in HPLC after 24 hours. Figure 8 shows Chromatogram for photodegraded sample

FORCED DEGRADATION PHOTO 24 HR (30 MCG)

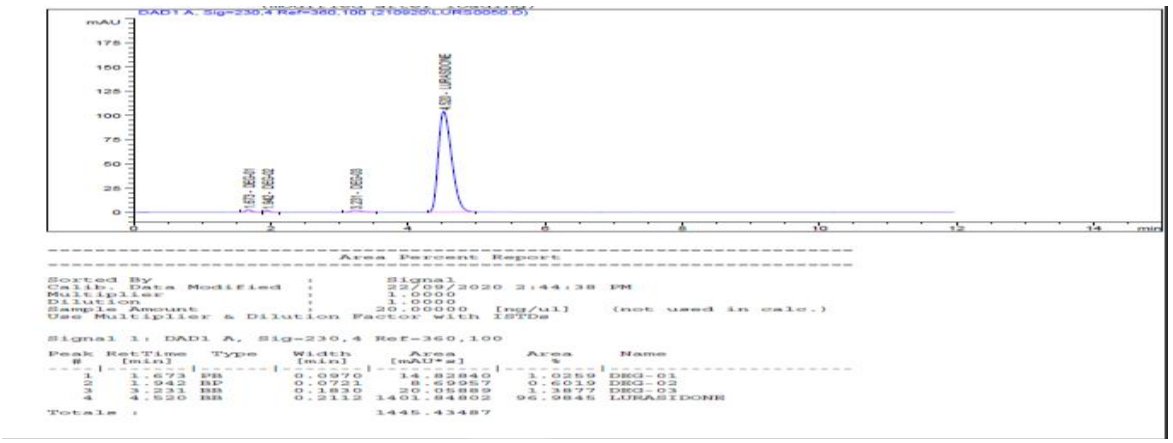
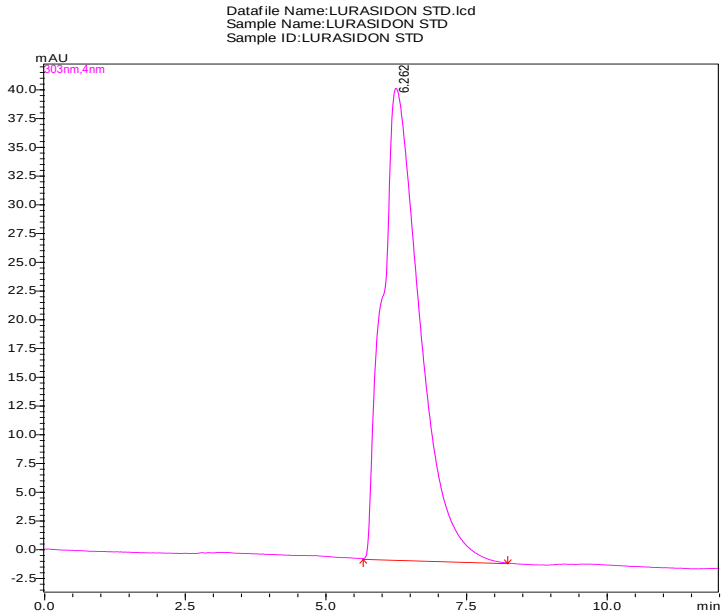

mAU
min
Area Percent Report
Sorted By : Signal
Calib. Data Modified : 22/09/2020 1:44:38 PM
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 20.00000 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
Signal 1: DAD1 A, Sig=230,4 Ref=360,100
Peak RetTime Type Width Area Area Name
[min] [min] [mAU*s] %
1 1.673 PB 0.0970 14.82840 1.0259 DEG-01
2 1.942 BP 0.0721 8.69957 0.6019 DEG-02
3 3.231 BB 0.1830 20.05889 1.3877 DEG-03
4 4.520 BB 0.2112 1401.84802 96.9845 LURASIDONE
Totals : 1445.43487

Figure 8: Chromatogram for photodegraded Lurasidone hydrochloride

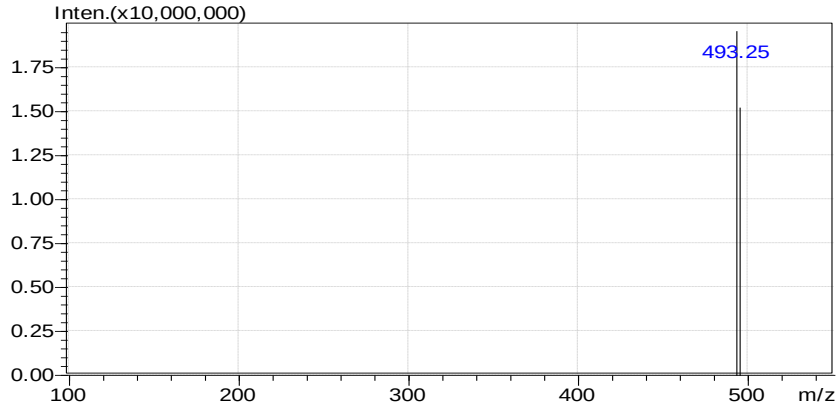
Lurasidone hydrochloride degraded in all the above conditions shows high degree of electivity of proposed HPLC method.

Forced degradants studies using LC-MS

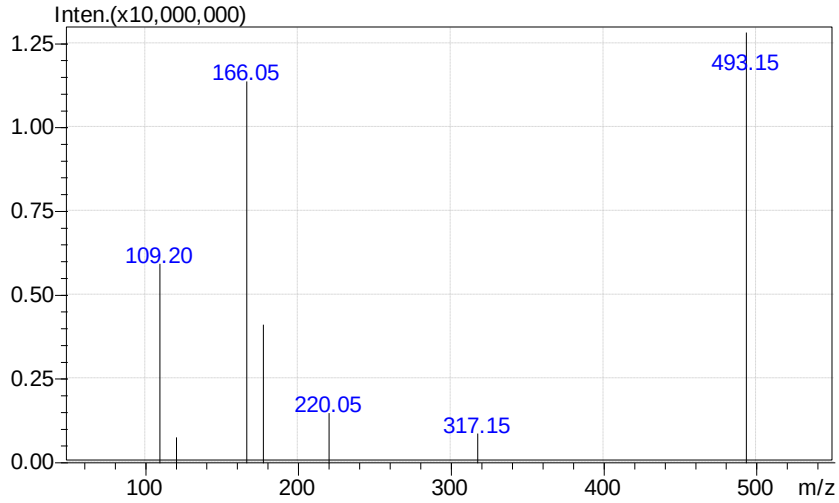
LC-MS chromatograph of standard Lurasidone hydrochloride solution 20ng/ µgL with optimised chromatographic conditions.



Peak#	Ret. Time	Area	Area%	Height
1	6.117	1379628	100.000	24965
Total		1379628	100.000	24965



Q1 Scan(E+) Ret. Time : [5.627->7.521]-[9.114->11.052] Scan# : [3555->4751]-



Product Ion Scan(E+) Precursor: 493.25 CE:-35.0 Ret. Time : [5.494->8.052]

The MS analysis of the acid degraded products in 0.1N hydrochloric acid, indicate presence of m/z of 133.15, 162.95, 195.95, 212.94, 217.05, 245, 261, 344.15, 351.60, 372.25, 383.35, 388.25, and 399.

MS Analysis of the alkali degraded products in 0.1N sodium hydroxide solution, indicate the presence of m/z of 190.80, 217.15, 226.65, 245.05, 261.20, 278.75, 294.35, 295.85, 330.30, 346.20, 351.45, 362.70, 383.45, 430.70 and 447.20.

The m/z that are seen in both acid and alkaline hydrolysis of Lurasidone are 217, 245, 261, 351 and 383. These are in accordance with the earlier reported studies indicating that the nano structured lipid carrier formulation does not significantly alter the molecular characteristics of the drug molecule.

There were negligible degradant in Hydrogen peroxide degraded product and no degradation detected in Neutral and photolytic degraded samples.

The results obtained from this validation study shows that method is precise, accurate, robust, specific, sensitive, cost effective and stability indicating. The method can be used in estimation of Lurasidone hydrochloride in bulk.

Optimized NLC batch details

Table 9: Optimized NLC batch

Ingredients	**%**
Glyceryl monostearate	3.2
Capryol-90	4.4
Tween 80	0.4
Lurasidone hydrochloride	1
Water	92

Based on DOE batches were taken to optimize the formula and process. No Probe sonication of NLC required. The NLC obtained by hot emulsification technique optimized process showed particle size of 36nm. So probe sonication step was dropped.

Table 10: Characterisation of optimised batch

Parameters	**Results**
Particle size	98.58 nm
Poly dispersibility index	0.355
Zeta potential	+18.8
Entrapment efficiency	96.7%
Assay	109.82%
SEM	Under analysis

Below SEM image of NLC formulation shows uniform NLC. Figure 9

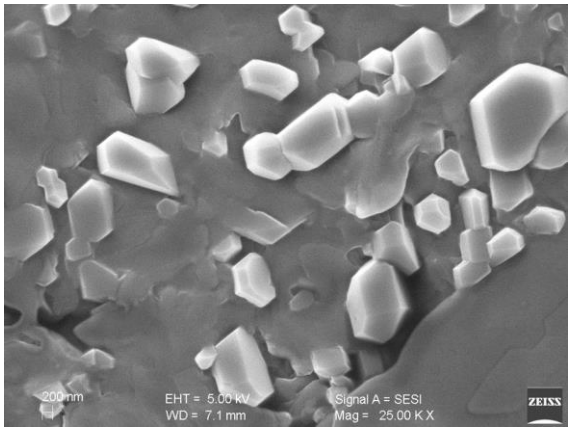


Figure 9: SEM image of NLC formulation

6 Month accelerated stability study of optimized batch complete. Stability samples, physical parameters satisfactory. Assay analysis ongoing. Results compilation in table 11 below

Table 11: Stability results of optimised batch

Parameters	**Initial**	**1M 40°C/75%RH**	**3M 40°C/75%RH**	**6M 40°C/75%RH**
Particle size(nm)	99.58	98.85	128.3	128.08
Poly dispersibility index	0.355	0.379	0.506	0.392
Zeta potential (mv)	+18.8	+20.6	+19.5	+20.80
Entrapment efficiency (%)	96.7%	94.74%	93.4%	94.68%
Assay (%)	109.82%	Under analysis		

Optimised *In-situ* gel NLC formulation details

Below formulation trials in table 12 were taken for in-situ gel formulation.

Table 12: In-situ gel formulation trials on Lurasidone hydrochloride NLC

Role	**Ingredients**	**F1**	**F2**	**F3**	**F4**
		% w/w			
API	Lurasidone hydrochloride	1	1	1	1
Solid lipid	Glyceryl monostearate	3.2	3.2	3.2	3.2
Liquid lipid and Co-surfactant	Capryol-90	4.4	4.4	4.4	4.4
Surfactant	Tween-80	0.4	0.4	0.4	0.4
Propyl paraben	Preservative	0.02	0.02	0.02	0.02
Gelling agent	Gellan gum	0.1	0.2	0.3	0.4
Mucoadhesive gelling agent	HPMC-K4 M	0.1	0.2	0.3	0.4
Solvent	Water	q.s 100	q.s 100	q.s 100	q.s 100

Procedure

To the NLC formulation, weighed quantities of propyl paraben, gellan gum and HPMC-K4 M were added under stirring using magnetic stirrer.

Gel formation in simulated nasal fluid and gelling time were recorded.

Below in table 13 is optimised in situ gel formulation finalised

Table 13 Optimised in-situ gel formulation

Ingredients	**Quantity (%)**
Glyceryl monostearate	3.2
Capryol-90	4.4
Tween 80	0.4
Lurasidone hydrochloride	1
Gellan gum	0.4
HPMC K4M	0.4

Propyl paraben	0.02
Water	q.s to 100

Characterization of in-situ gel

Gelation study

In-situ gel formulation and simulated nasal fluid SNF (1:1v/v ratio) were mixed on magnetic stirrer. The gelation point was determined when the magnetic bar stopped moving due to gelation

Viscosity study

The viscosity of nasal formulation before and after gelation was determined using Brookfield Rheometer R/S-CPS +1600

Assay: Assay of in situ-gel formulation is under analysis using developed HPLC method.

Below table 14 shows observed in-situ gel characterisation parameters.

Table 14: Characterisation parameters of in-situ gel formulation

Parameters	Observed values
Gelling capacity	Gel formed in 60 seconds and remain stable for 2 hours. ++
Viscosity	54 cps, 100 cps
Assay	Under analysis

PhD Research work publication details as in Table 15. One review article published.

Publication details

Name of the authors	**Title of the article / research paper**	**Name of the Journal**	**Publishing Authority**	**Volume / Page No.**	**Date / Year (MM-YY)**	**Indexed**	**Impact Factor**
Tanvi Kadam Surendra Agrawal	A Short Review on the Important Aspects Involved in Preparation, Characterization and Application of Nanostructured Lipid Carriers for Drug Delivery	Current Nanomedicine	Bentham Science	Volume 10, Number 3, 2020, pp. 188-207(20)	September 1, 2020	Scopus	0.8

Manuscripts ready for publications/communicating to journals.

2. Tanvi Kadam, Dr Surendra Agrawal, Dr Saritha Shetty

Pharmacokinetics Studies of Lurasidone Hydrochloride in Nano Structured Lipid Carrier Formulation

3. Tanvi Kadam, Dr Surendra Agrawal, Dr Saritha Shetty

Reverse Phase HPLC Validated Method for estimation of Lurasidone Hydrochloride in bulk and novel formulations and its degradation studies using HPLC and LC-MS

4. Tanvi Kadam, Dr Surendra Agrawal, Dr Saritha Shetty

Development of nanostructured lipid carriers of Lurasidone for intranasal delivery: Approach to enhance bioavailability.

Characterization of the forced degradants using LC-MS.

Procedure

Preparation of Mobile phase/diluent or buffer solution

In a 100 ml volumetric flask 60 ml of Methanol and 40 ml of buffer solution consisting of 0.1% orthophosphoric acid in water was taken. This solution was sonicated for 10 minutes and filtered through a 0.45µ membrane filter. This formed the mobile phase or diluent.

Preparation of Lurasidone hydrochloride stock / standard solution

10mg of Lurasidone hydrochloride was accurately weighed and transferred to 10ml volumetric flask. To this diluent/mobile phase was added and sonicated for 10 mins to dissolve the drug. Volume was made up to 10ml with diluent. The solution was filtered through 0.45µ filter and filtrate to form Lurasidone hydrochloride stock solution of 1000µg/ml.

Acid degradation studies in 0.1N hydrochloric acid.

To 0.3 ml of Lurasidone hydrochloride stock solution, add 5 ml of 0.1N HCL and make up the volume to 10 mL with mobile phase. Reflux at 70^{o}C up to 6 hours. 6 hours sample send for LC-MS for degradant analysis.

Alkali degradation studies in 0.1N sodium hydroxide solution.

To 0.3 ml of Lurasidone hydrochloride stock solution, add 5 ml of 0.1N NaOH solution and ma***k***e up the volume to 10 mL with mobile phase. Reflux at 70^{o}C up to 6 hours. 6 hours sample send for LC-MS for degradant analysis.

Neutral degradation studies in water.

To 0.3 ml of Lurasidone hydrochloride stock solution, added 5 ml of water and made up the volume to 10 mL with mobile phase. Reflux at 70^{o}C up to 6 hours. 6 hours sample send for LC-MS for degradant analysis.

Peroxide degradation studies in Hydrogen peroxide (H_2O_2) solution.

To 0.3 ml of Lurasidone hydrochloride stock solution, added 5 ml of H_2O_2 solution and made up the volume to 10 mL with mobile phase. Reflux at 70^{o}C up to 6 hours. 6 hours sample send for LC-MS for degradant analysis.

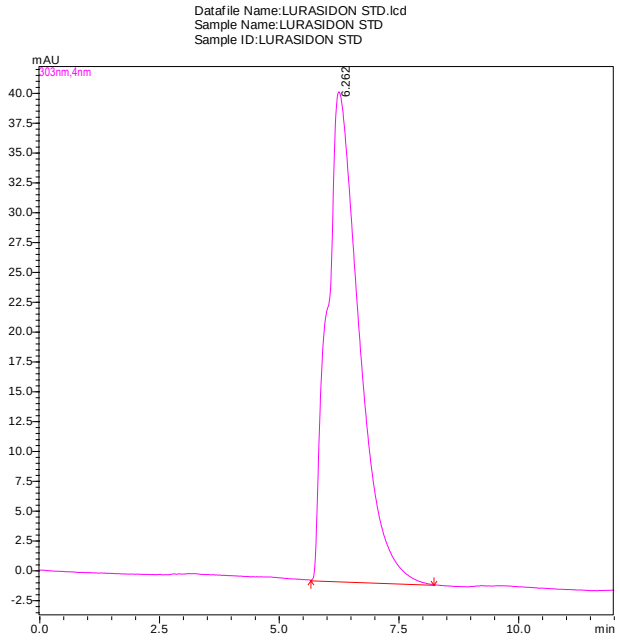
Photolytic degradation

Photodegraded Lurasidone hydrochloride 30 MCG sample in 10 mL Mobile phase was injected in HPLC after 24 hours. Figure 8 shows Chromatogram for photodegraded sample

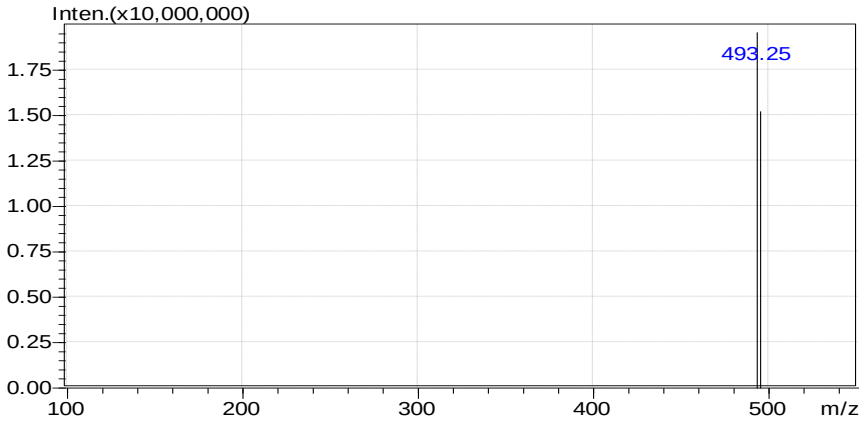
The m/z for Lurasidone was 493.25. The precursor ion of m/z 493.25, at collision energy of 35 eV gave m/z peaks at 109.20, 166.05, 220.05 and 317.15 (Fig 10)

The LC-MS analysis of the degraded products in acid indicate presence of m/z of 133.15, 162.95, 195.95, 212.94, 217.05, 245, 261, 344.15, 351.60, 372.25, 383.35, 388.25, and 399.

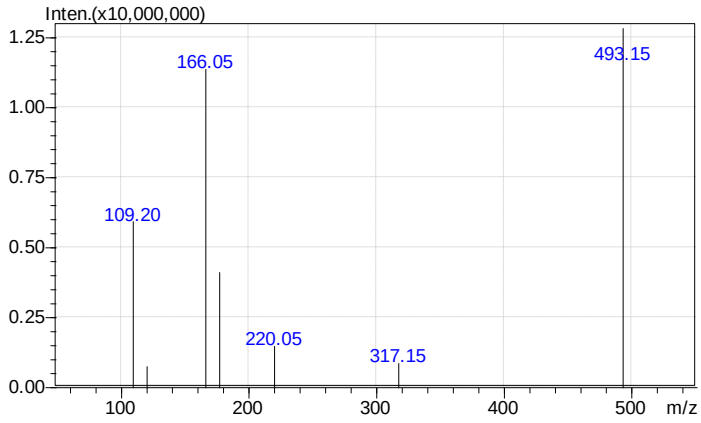
LC-MS Analysis of the degraded products in alkali indicate the presence of m/z of 190.80, 217.15, 226.65, 245.05, 261.20, 278.75, 294.35, 295.85, 330.30, 346.20, 351.45, 362.70, 383.45, 430.70 and 447.20.



Peak#	Ret. Time	Area	Area%	Height
1	6.117	1379628	100.000	24965
Total		1379628	100.000	24965



Q1 Scan(E+) Ret. Time : [5.627->7.521]-[9.114->11.052] Scan# : [3555->4751]-



Product Ion Scan(E+) Precursor: 493.25 CE:-35.0 Ret. Time : [5.494->8.052]

Figure 10: LC-MS chromatograph of standard Lurasidone hydrochloride solution 20ng/ µgL with optimised chromatographic conditions.

Pharmacokinetic study

Methodology

After the approval from the Institutional Animal Ethics Committee (Protocol No. PES's RTBCOP/IAEC; Clear 2021 R 99). Albino Wistar rats of 200-250 g weight were used for pharmacokinetic study. The animals were kept fasted overnight with free access to water throughout the experimental period. The 0.81 mg/kg dose of API and optimized formulation were administered to the Wister male rats (n= 10) except normal group by nasal route. Collected the 0.25 ml of blood samples from the retro-orbital plexus mild anesthesia with isoflurane in micro-centrifuge tubes containing 10 µl of EDTA at the predetermined time (from 0.5-24 hrs). The plasma was collected from the samples by centrifugation process at 4000 rpm for 10 min. The obtained plasma samples (200 µl) were stored at -20 °C till the examination of drug concentration. The aliquot was then used for HPLC analysis. The pharmacokinetic parameters were evaluated by a non-compartmental model with the aid of the add-in program PKSolver. All values were expressed as their mean ± SD. Differences were regarded to be significant as the level of $p < 0.05$.

Tissue bio-distribution study

To investigate the bio-distribution of free API and NLC, 6 male Wistar rats (200 g) were arbitrarily divided into two groups (six in each). Free API and nanoliposome were administered by nasal route at dose 0.81 mg/kg API equivalent respectively. Rats were sacrificed by excess anesthesia after 24 h following administration of the dose. The brain was harvested and weighed. Two hundred milligram of tissue was homogenized with methanol (5 mL), centrifuged at 20,000 rpm and 4°C for 20 min. Later, the filtered supernatant was introduced into the HPLC system to determine API concentration.

Pharmacokinetic Study

Results

Free drug and NLC formulation were administered via nasal route with dose of 0.81 mg/kg (equivalent to free drug). The comparative pharmacokinetic parameters between free API and NLC containing lurasidone hydrochloride rats were calculated. The concentration of drug was found to be 4085.11 mcg/ml in blood when the drug administered in the form of NLC as compared to free drug (3260.98 mcg/ml). Optimized formulation showed good increase in the Cmax. (Figure 9, Table16)

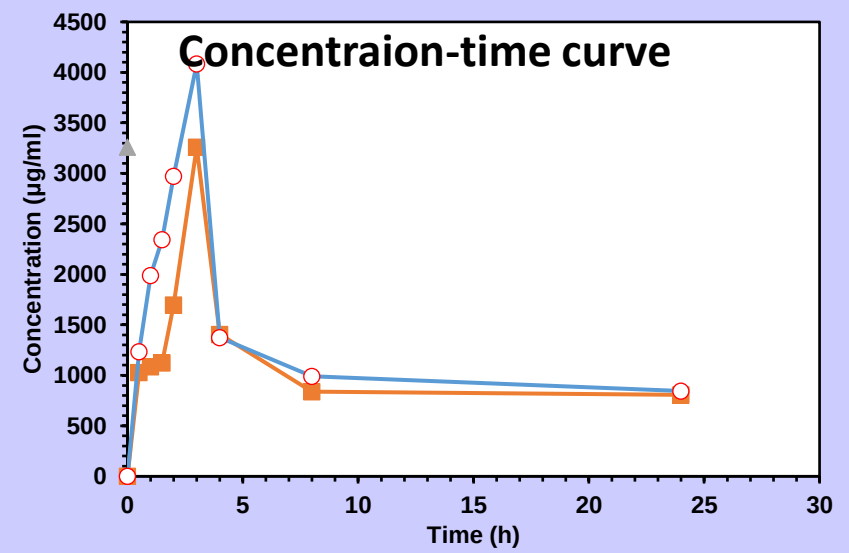


Figure 9: Concentration time curve of plain Lurasidone hydrochloride and NLC formulation in rat plasma

Table 16: Pharmacokinetic parameters of NLC formulation and Plain Lurasidone hydrochloride and NLC formulation

Parameter	Unit		Value
		Free drug	NLC
Lambda_z	1/h	0.044584392	0.020273405
t1/2	h	15.54685733	34.18997288
Tmax	h	3	3
Cmax	µg/ml	3260.983674	4085.111052
Tlag	h	0	0
Clast_obs/Cmax		0.247158607	0.206496647
AUC 0-t	µg/ml*h	24483.83422	29187.07255
AUC 0-inf_obs	µg/ml*h	42561.46511	70796.34929

AUC 0-t/0-inf_obs		0.575258257	0.412268045
AUMC 0-inf_obs	µg/ml*h^2	1089073.03	3325757.672
MRT 0-inf_obs	h	25.58824108	46.97640069
Vz/F_obs	(mg)/(µg/ml)	0.00042686	0.000564349
Cl/F_obs	(mg)/(µg/ml)/h	1.90313E-05	1.14413E-05

Results Bio-distribution study

The tissue distribution of lurasidone from NLC formulation was determined after administration of the optimized formulations at a dose of 0.81 mg/kg. The average concentration of lurasidone in NLC was found to be 1114.65 mcg/g in brain. The concentration of drug in brain when administered in free form was found to be 27.49 mcg/g. The NLC exhibited better drug targeting to brain as compared to free drug form. As expected, in NLC formulation the nano size vesicles were well absorbed and targeted brain tissue. (Figure 10)

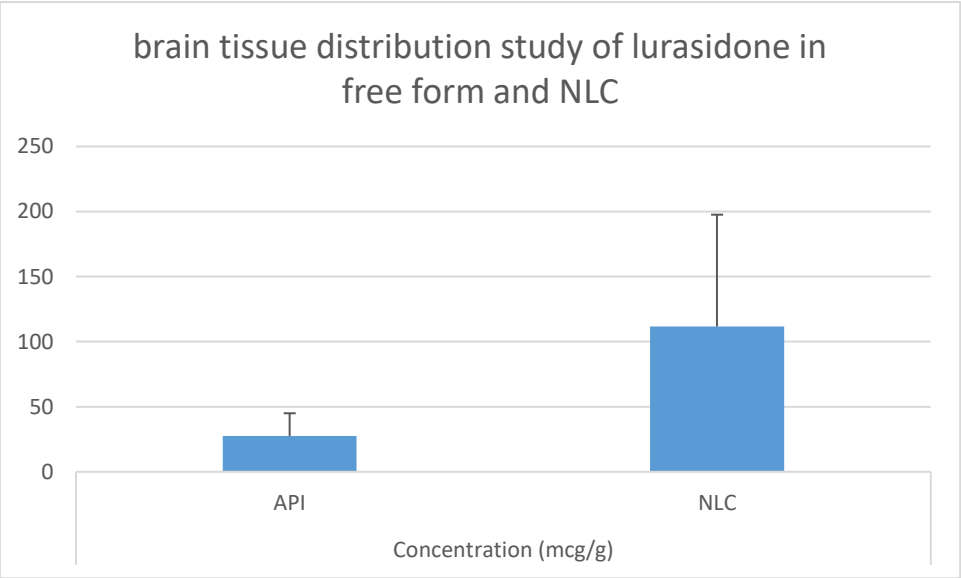


Figure 10: Brain tissue distribution of plain Lurasidone hydrochloride and NLC formulation in rats

RP-HPLC BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF LURASIDONE HYDROCHLORIDE IN HUMAN PLASMA

RP-HPLC developed and validated analytical method for determining Lurasidone hydrochloride in bulk drug and NLC formulation was applied to check bioanalysis of drug and NLC formulation in human plasma.

Differences between developed analytical method and bioanalytical method, is as shown in

table 17 below

Table 17: Comparison of validated RP-HPLC analytical and Bio-analytical methods

Parameters	**Developed analytical method**	**Developed bio analytical method**	**Observations Developed method vs reported method**
HPLC column	Fortis $C_{18,}$ Column (4.6 x 100 mm)	Fortis $C_{18,}$ Column (4.6 x 250 mm)	
Particle size	2.5 μm	5.0 μm	Finer particle size gave better resolution
Optimized mobile phase	Methanol and water containing 0. 1% Ortho Phosphoric Acid in 60:40 (v/v) ratio	Methanol and water containing 0. 05% Ortho Phosphoric Acid pH 3.0 in 65:35 (v/v) ratio	Lower concentration of methanol and hence more cost-effective method
flow rate	0.7 ml/min	0.8 ml/min	Lower flow rate
Sample volume	20 μl	20 μl	
HPLC system	Agilent Tech. Gradient System with Auto injector using UV (DAD) G13148 detector	Agilent Tech. Gradient System with Auto injector using UV (DAD) G13148 detector	
Detection wavelength	230 nm	230 nm	

Bioanalytical method development

Diluetant = Methanol +0.05 % OPA (100ML WATER) (65+35)

Buffer = 0.05 % OPA (100ML WATER)

Preparation of Stock Standard Solution:

Preparation of the individual standard preparation

10 mg Amount of standard drug was mixed with 10 ml methanol to + 2 ml of human plasma (untreated) then vertically Shaked for 30 min then centrifuged at 5000 rpm for 1h and filtered using membrane filters to get clear organic solution. The solution was filled in the sample vials of HPLC and loaded on to HPLC for Run.

Preparation of the LURASIDONE sample solution

Sample solution preparation

Blood samples are collected from human and then centrifuged at 5000rpm for 1h to separate the plasma from blood. Then the separated plasma was mixed with water then loaded on to the HPLC for Run.

Standard Sample Preparation-

1) Std LURASIDONE 10 mg in 10 ml diluentant = 1000 µgm/ml --- STOCK I
2) Take 0.1 from stock I . and make vol. with mobile phase 10 ML
   = 10 µgm/ml LURASIDONE
3) Take 0.2 from stock I . and make vol. with mobile phase 10 ML =
   = 20 µgm/ml LURASIDONE
4) Take 0.3 from stock I . and make vol. with mobile phase 10 ML
   = 30 µgm/ml LURASIDONE
5) Take 0.4 from stock I . and make vol. with mobile phase 10 ML
   = 40 µgm/ml LURASIDONE
6) Take 0.6 from stock I . and make vol. with mobile phase 10 ML
   = 50 µgm/ml LURASIDONE

NLC formulation solution Preparation:-

Take 0.1 ml of NLC formulation in 10 ml Mobile Phase i.e. = 1000 µg/ml NLC solution STOCK II

TAKE 0.5 ML FROM STOCK II AND MAKEUP VILUME 10 ML WITH MP=50 µgm/ml

FOR ACCURACY solution Preparation:-

TAKE 10 µgm/ml NLC SOLUTION FOR AUURACY

10 X 80 % = 8µgm/ml STD (0.08 ML)

10 100 % = 10µgm/ml STD (0.1 ML)

10 X120 % = 12µgm/ml STD (0.12 ML)

80 % = 0.1 ML NLC SOLUTION and ADD 8µgm/ml std
AND MAKE UP VOL 10 ML WITH MOBILE PHASE

100 % =0.1 ML NLC SOLUTION and ADD 10 µgm/ml STD.
AND MAKE UP VOL 10 ML WITH MOBILE PHASE

120 %= 0.1 ML NLC SOLUTION and ADD 12 µgm/ml STD.
AND MAKE UP VOL 10 ML WITH MOBILE PHASE

HPLC Details

Pump unit = G1310A ISOPUMP

Detector- G-13148 (DAD)

MAXIMUM PRESSURE = 400 BAR

DISCHARGE Rate = 0.001 to 5 ml

Pressure limit range = 400 bar

Pressure display accuracy =5 %

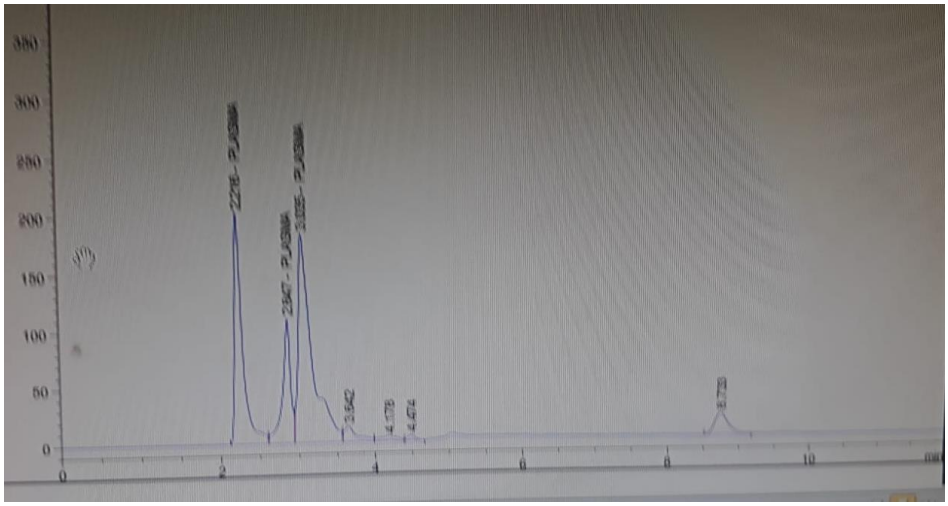


Figure 11: RP-HPLC graph of blank human plasma

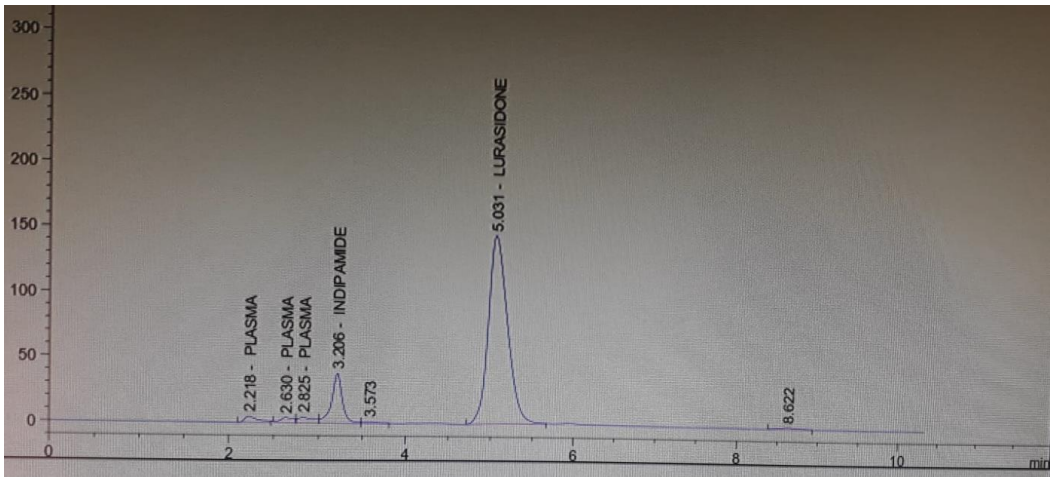
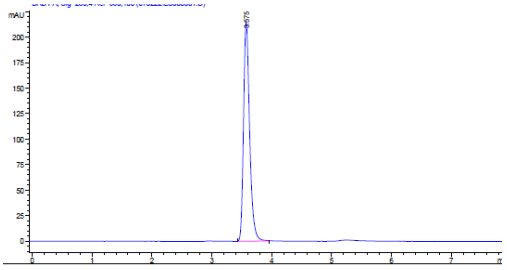
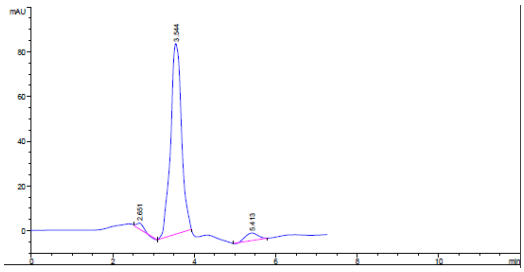
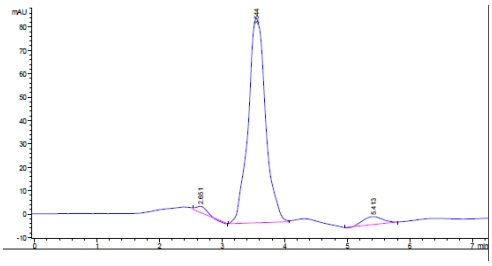
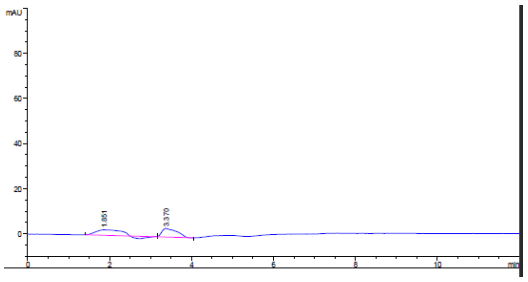
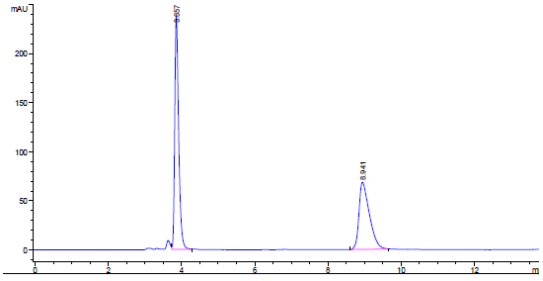
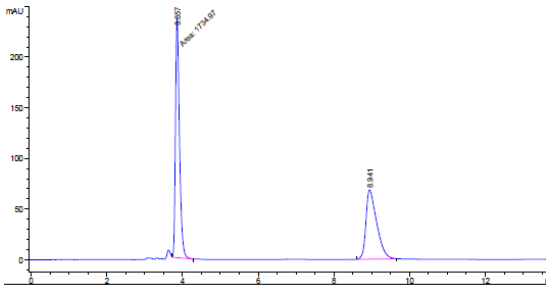
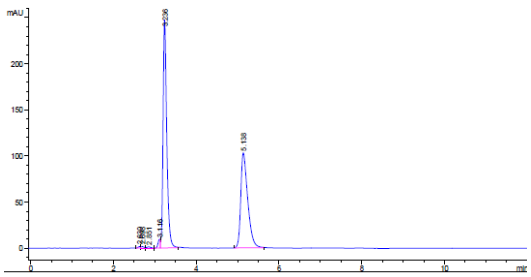
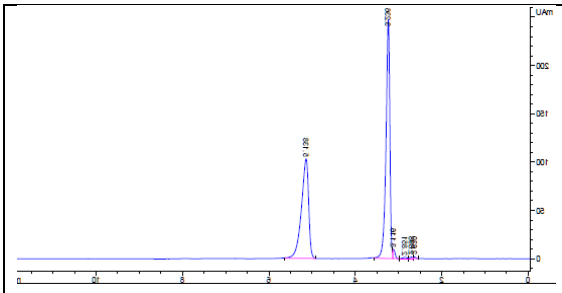


Figure 13: RP-HPLC graph of blank human plasma, Internal standard and Lurasidone hydrochloride drug.

LURASIDONE-Trial 01 80MEOH + 20 WATER 9(20 MCG)0.7 	LURASIDONE-2 60MEOH + 40 WATER 9(20 MCG)0.7 
LURASIDONE-Trial 2 60MEOH + 40 WATER 9(20 MCG)0.7 	LURASIDONE-Trial 3 50 MEOH + 50 WATER 9(20 MCG)0.7 
TRIAL 4 LURASIDONE(20 mcg) + 10 mcg IS-60 MEOH + 40 (0. 05%OPA) 0.7 	TRIAL 5 LURASIDONE(20 mcg) + 10 mcg IS-60 MEOH + 40 (0. 05%OPA) 0.7 
TRIAL 6 LURASIDONE(20 mcg) + 10 mcg IS-65 MEOH + 35 (0. 05%OPA) 0.7	TRIAL 7 LURASIDONE(20 mcg) + 10 mcg IS-65 MEOH + 35 (0. 05%OPA) 0.7 



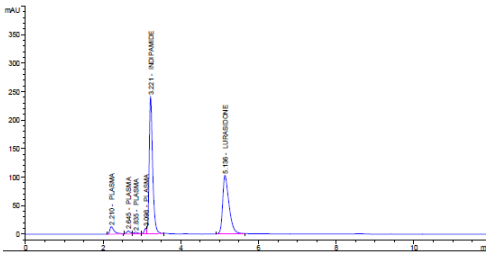
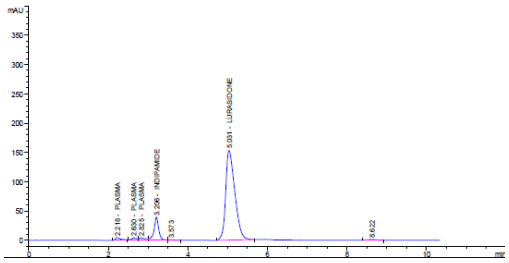
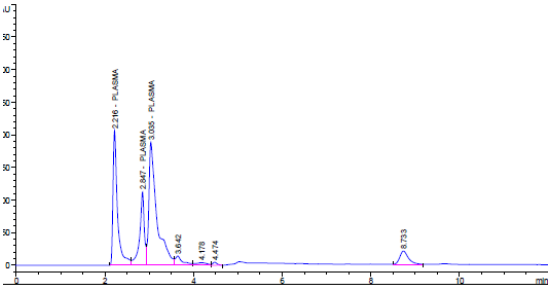
TRIAL 8 (Final) LURASIDONE(20 mcg) + 10 mcg IS+PLASMA-65 MEOH + 35 (0.05%OPA) 0.7	TRIAL 9 LURASIDONE(20 mcg) + 10 mcg IS+PLASMA-65 MEOH + 35 (0.05%OPA) 0.8 FINAL
	
BLANK PLASMA 0.8 ML FLOW 65 MEOH+ 35 (0.05 % OPA_)250X4.6 MM AND 5 MIC	
	

Figure 14: Chromatograms of different mobile phases trials, columns width and particle size packings and flow rate.

The chromatographic conditions from the bio analytical optimisation studies using various set parameters were selected. Retention time of Lurasidone hydrochloride was obtained at 5.031 ± 0.02 mins with mobile phase consisting of Methanol + buffer containing 0.05% Orthophosphoric acid in water in ratio of 65:35 (%v/v). The column selected was 100 x 250 mm with particle size packing 5.0µ. The detection wavelength was set to 230 nm with a flow rate of 0.8ml/min.

METHOD VALIDATION

The optimized method was validated [8,9] as per ICH guidelines [10]

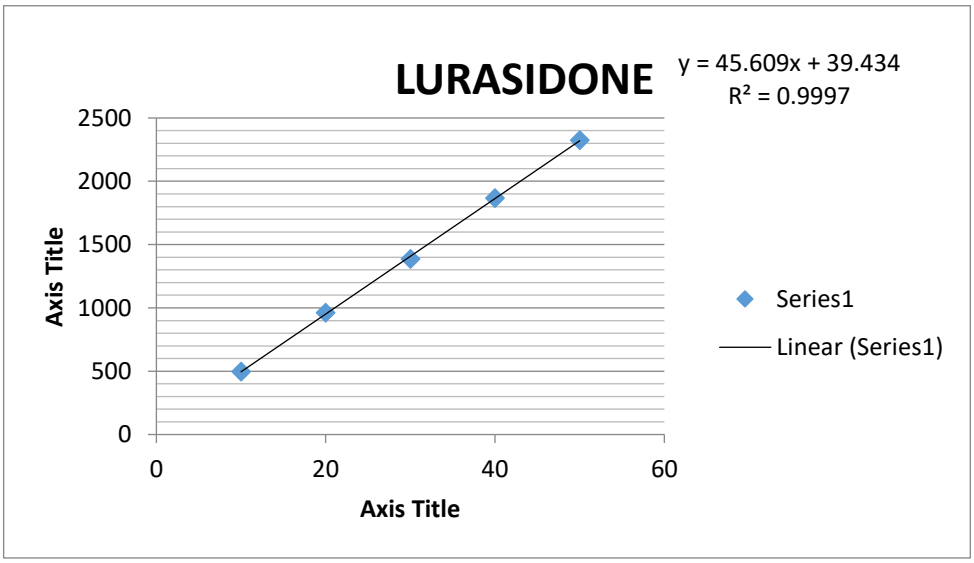
Linearity

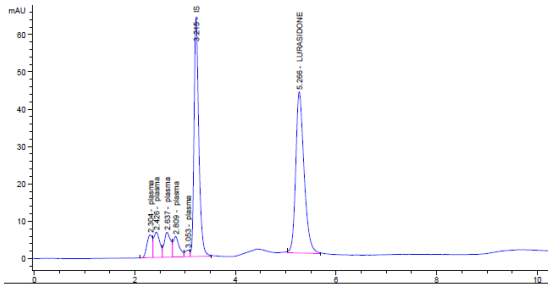
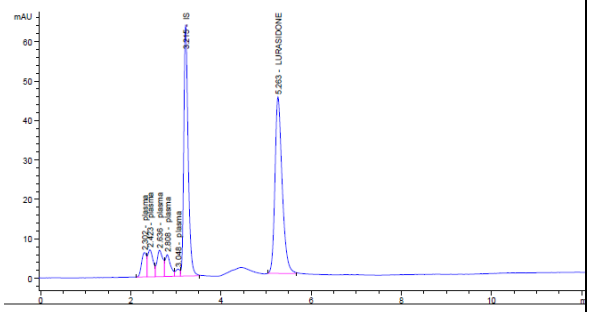
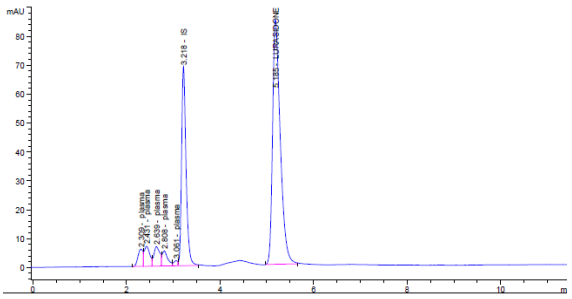
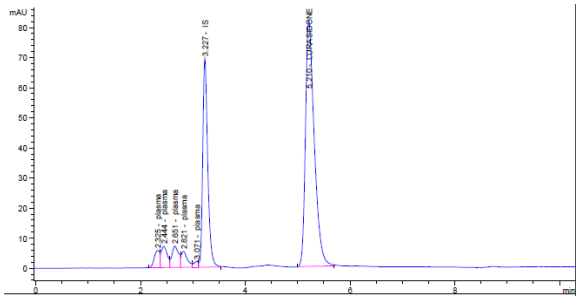
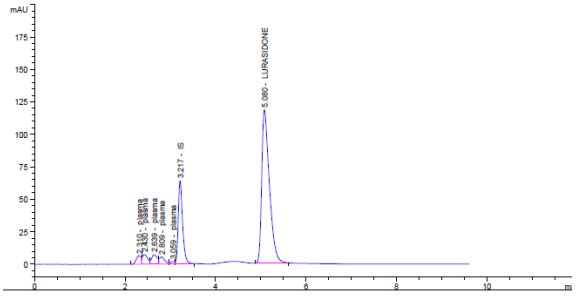
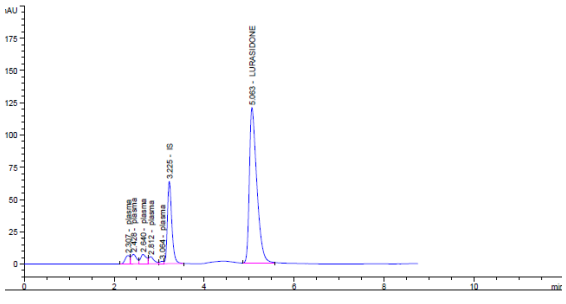
The linearity of bioanalytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. Linearity of Lurasidone hydrochloride was studied for 10, 20, 30, 40 and 50 µg/ml concentrations. These solutions were run in HPLC system for 10 mins at flow rate of 0.8ml/min. The Chromatographs shows concentration of Lurasidone hydrochloride on x axis and peak area on y axis. The peak area response vs concentration prepared was used for concentration of calibration curve. The calibration curve was found to be accurately fit with very high correlation of 0.9997. The regression equation was found to be y = 45.609x +39.434 (Fig 15). The linearity study results were given in Table 18. Figure 15 shows Linearity calibration curve and Figure 16 shows Linearity chromatographs of Lurasidone hydrochloride.

Table 18: Linearity study results of Lurasidone hydrochloride

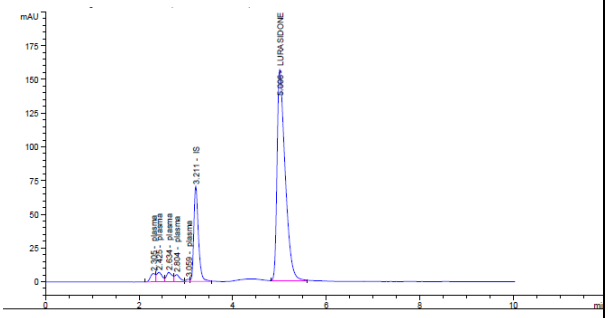
Sr No.	**Conc**	**Area I**	**Area II**	**Mean Area**	**SD**	**%RSD**
1	10	497.2791	495.9972	496.64	0.91	0.18
2	20	967.6635	956.6237	962.14	7.81	0.81
3	30	1392.787	1382.0205	1387.40	7.61	0.55
4	40	1870.642	1867.9892	1869.32	1.88	0.10
5	50	2338.8874	2313.0856	2325.99	18.24	0.78
				Avg. SD	**7.29**	

Figure 15: Linear calibration curve of Lurasidone hydrochloride

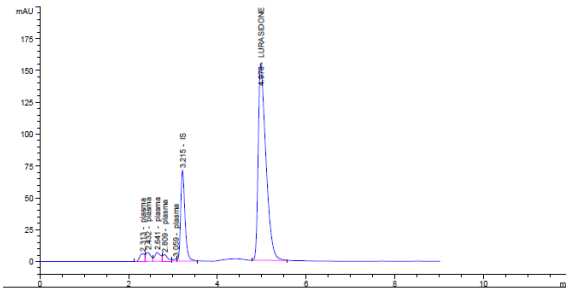


Linearity 10 mcg-01 	Linearity 10 mcg-02 
Linearity 20 mcg-01 	Linearity 20 mcg-02 
Linearity 30 mcg-01 	Linearity 30 mcg-02 

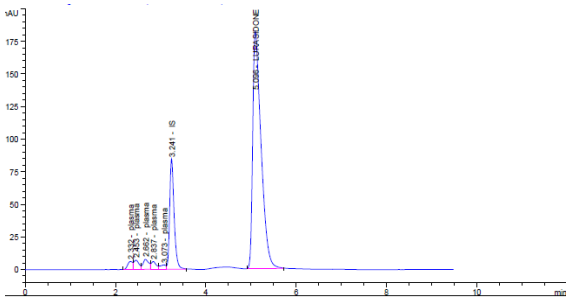
Linearity 40 mcg-01



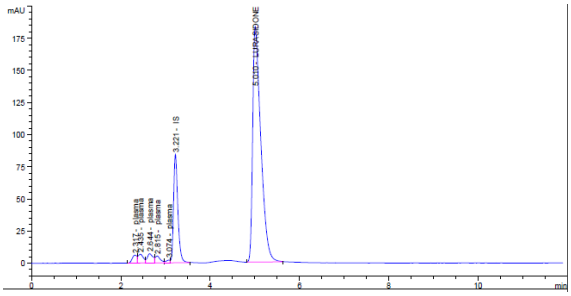
Linearity 40 mcg-02



Linearity 50 mcg-01



Linearity 50 mcg-02



Linearity overlay

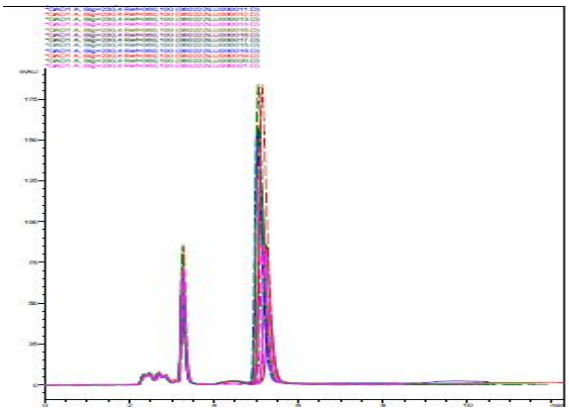


Figure 16 Linearity chromatographs of Lurasidone hydrochloride

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ values determines the sensitivity of the proposed method. LOD and LOQ are calculated from Standard deviations (7.29) and slope (45.6) determined by calibration curve in Linearity. Table 19 shows LOD and LOQ values.

Table 19: LOD and LOQ of Lurasidone hydrochloride

LOD= 3.3 X Avd.SD/ Slope

24.05453

0.527512

LOQ= 10X Avd SD/Slope

72.89253

1.59852

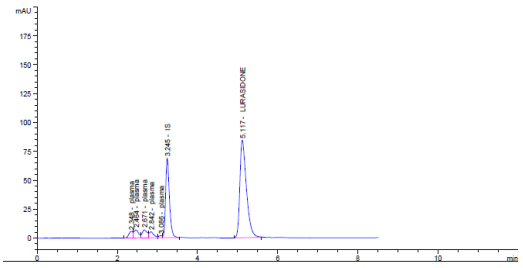
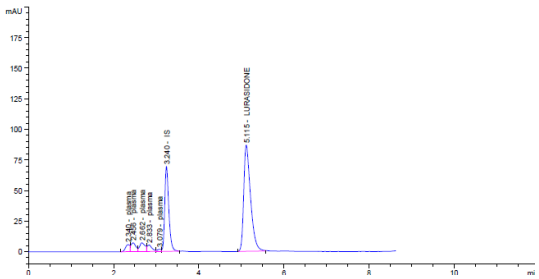
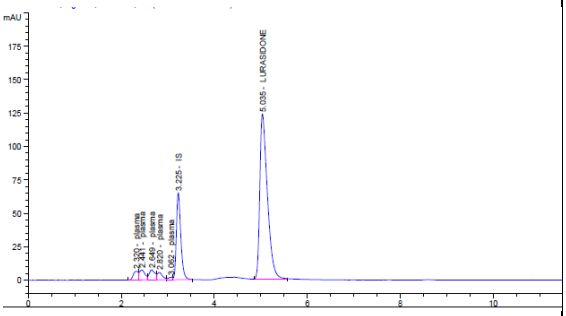
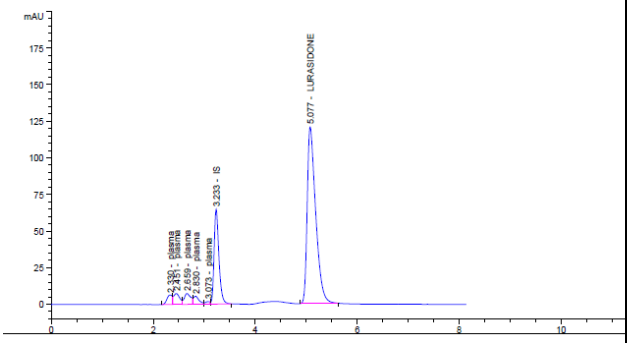
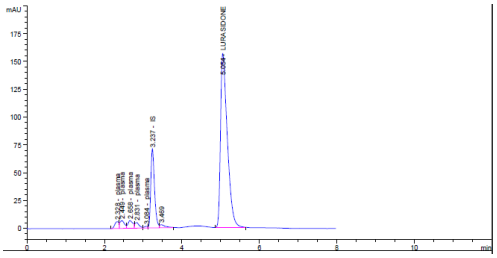
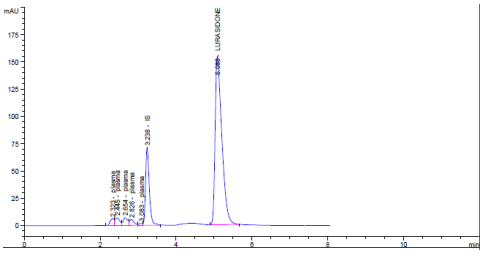
Precision

The precision of an analytical method determines the closeness of a series of individual analyte measurements applied repeatedly to multiple aliquots of the same sample. It is calculated as a Relative Standard Deviation (RSD). The intraday (same day) precision study was carried out by estimating the area peak responses of Lurasidone hydrochloride standard solution for 20, 30 and 40µg/ml. Each concentration was repeated two times. So total 9 estimations on same day. The interday (2 different days) precision study was carried out by estimating the area peak responses of Lurasidone hydrochloride standard solution for 20, 30 and 40µg/ml. Each concentration was repeated two times. So total 6 estimations on next day. Intraday and interday results in Table 20 depicts that method is precise. Figure 17 shows interday and intraday precision chromatographs

Table 20: Intraday and Interday precision for Lurasidone hydrochloride

Sample No.	Conc. µg/ml	Peak area of Lurasidone hydrochloride Interday	Mean	%RSD	Peak area of Lurasidone hydrochloride Intraday	Mean	%RSD
1	20	960.3286	964.17	0.56	967.667	965.38	0.34
2		968.0177			963.09		
1	30	1398.1336	1397.36	0.08	1402.6032	1403.12	0.05
2		1396.5774			1403.6428		

1	40	1907.8116	1903.99	0.28	1898.9061		0.19
2		1900.166			1893.7841	1896.35	

PRECISION 20 mcg (Interday)-01	PRECISION 20 mcg (Interday)-02
	
PRECISION 30 mcg (Interday)-01	PRECISION 30 mcg (Interday)-02
	
PRECISION 40 mcg (Interday)-01	PRECISION 40 mcg (Interday)-02
	
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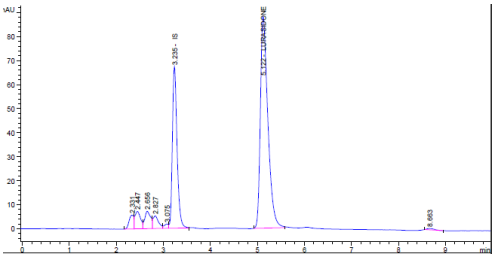
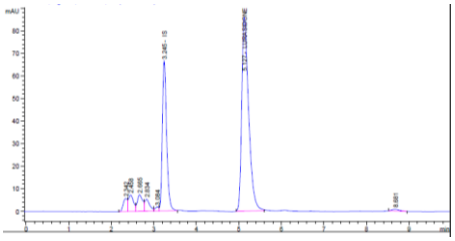
	
PRECISION 30 mcg (Intraday)-01	PRECISION 30 mcg (Intraday)-02

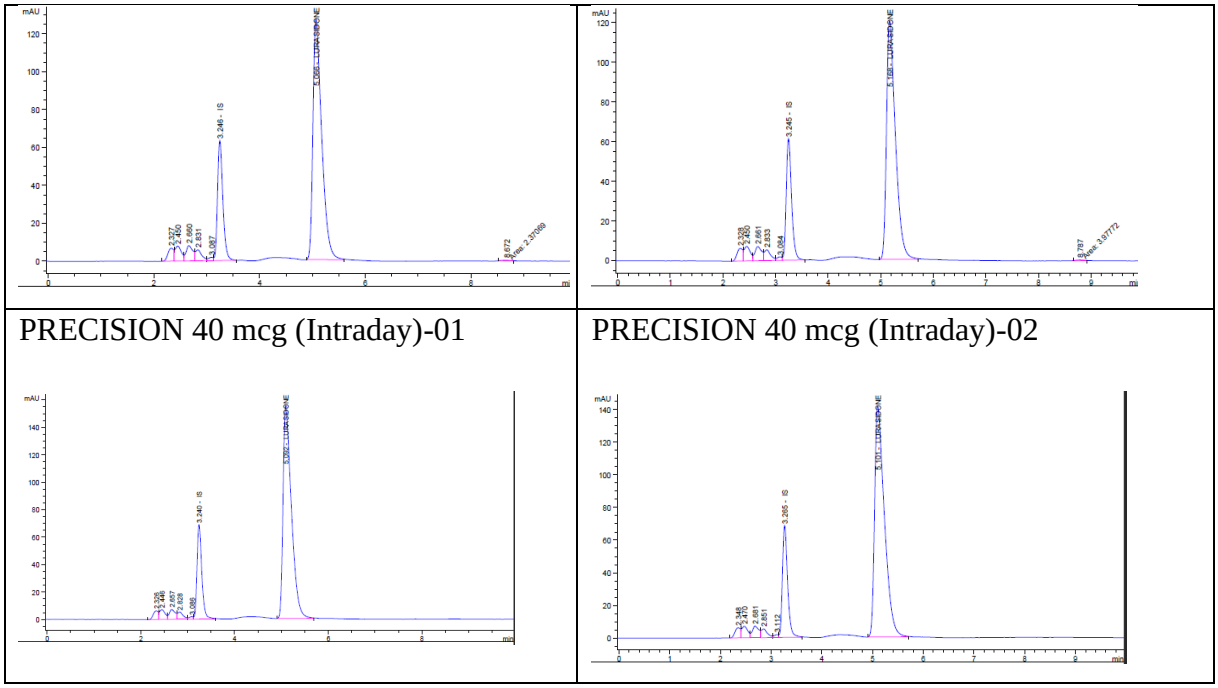


Figure 17: Interday and intraday precision chromatographs

Repeatability/System suitability

Repeatability was determined by injecting 20 µg/ml two times. Corresponding peak areas of the chromatograms generated were measured and %RSD calculated. The method is precise if %RSD less than 2%. Table 21 shows system repeatability results. Since %RSD is 0.32, the method is precise. Figure 18 shows repeatability chromatographs.

Table 21: Repeatability results

Sr No.	Conc. µg/ml	Area I	II	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	20	966.538	962.209	964.37	20.28	101.42	3.06	0.32

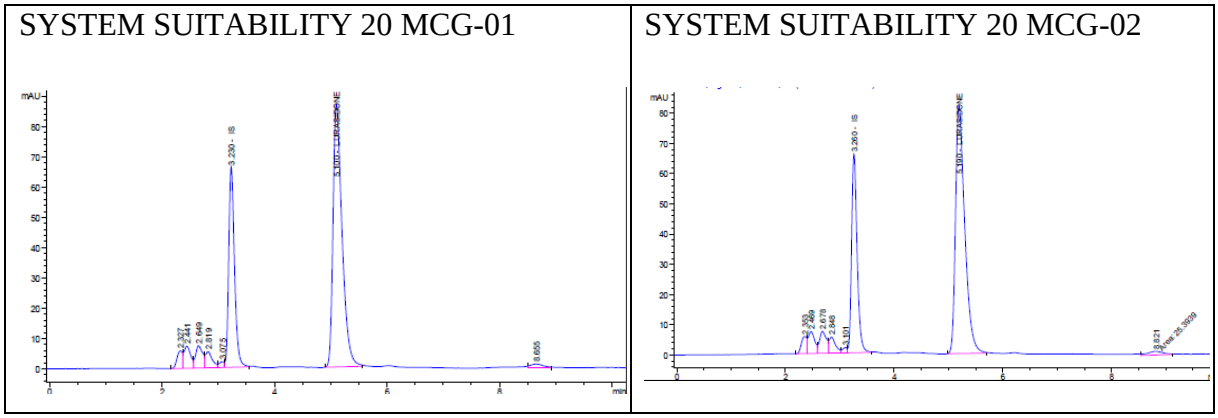


Figure 18 repeatability chromatographs

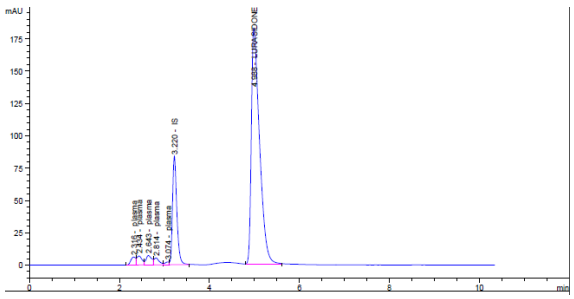
Assay

Assay was determined by repeating 50 µg/ml . Table 22 shows assay data and Figure 19 shows Chromatograph of Lurasidone hydrochloride.

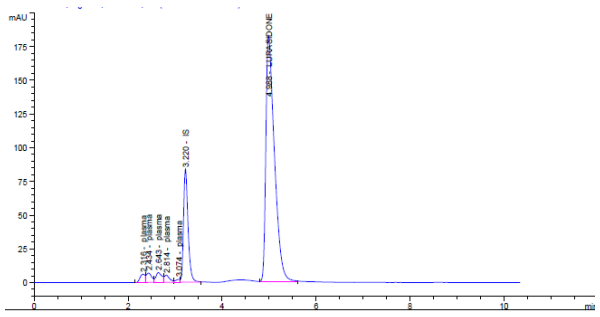
Table 22: Assay results

Sr No.	Conc. µg/ml	Area I	II	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	50	2279.891	2278.112	2279.00	49.11	98.23	0.055	0.056

ASSAY- 50 mcg-01



ASSAY- 50 mcg-02



ASSAY- Overlay

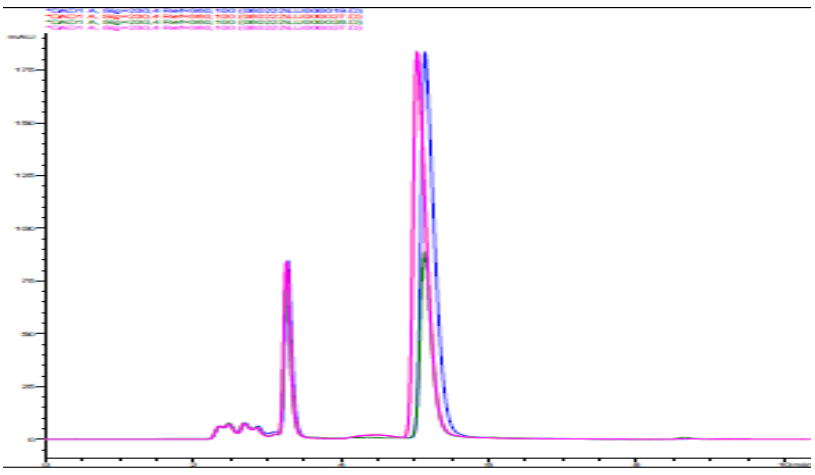


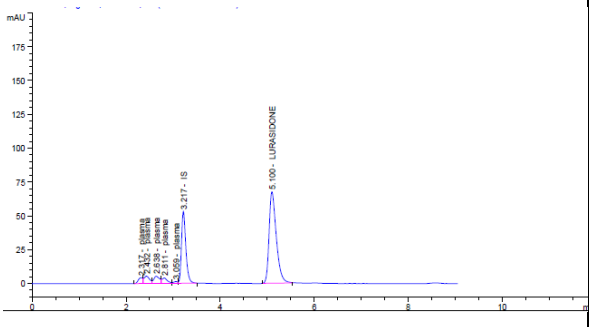
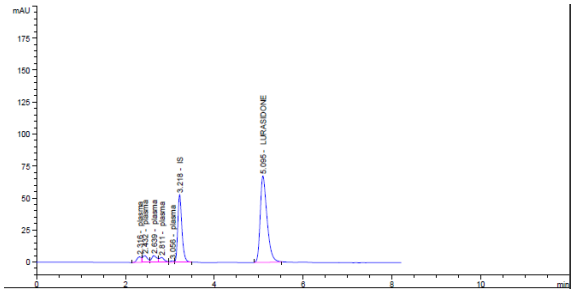
Figure 19 Assay chromatographs

Accuracy

The accuracy of the analytical method determines how close the test results are with actual values. Accuracy was done by recovery method. In this percent recovery of the analyte was determined. The recovery of standard solution of Lurasidone hydrochloride (10µg/ml) was estimated at two different levels (80%, 100% and 120%). Each level was recovered in 3 consecutive series. Percent recovery in Table 23 shows that method is accurate. Figure 20 shows related chromatographs.

Table 23: Percent recovery of Lurasidone hydrochloride

Sample No.	% spike level	Amount of Lurasidone hydrochloride spiked (µg/ml)	Amount of Lurasidone hydrochloride recovered (µg/ml)	% Recovery	Mean % recovery	SD	%RSD
1	80	8	7.83	97.89	97.70	0.27	0.27
2		8	7.80	97.51			
1	100	10	9.755776316	97.56	97.71	0.22	0.22
2		10	9.78627193	97.86			
1	120	12	11.8651864	98.88	98.52	0.50	0.51
2		12	11.77998026	98.17			

ACCURACY 80%-01	ACCURACY 80%-02
	
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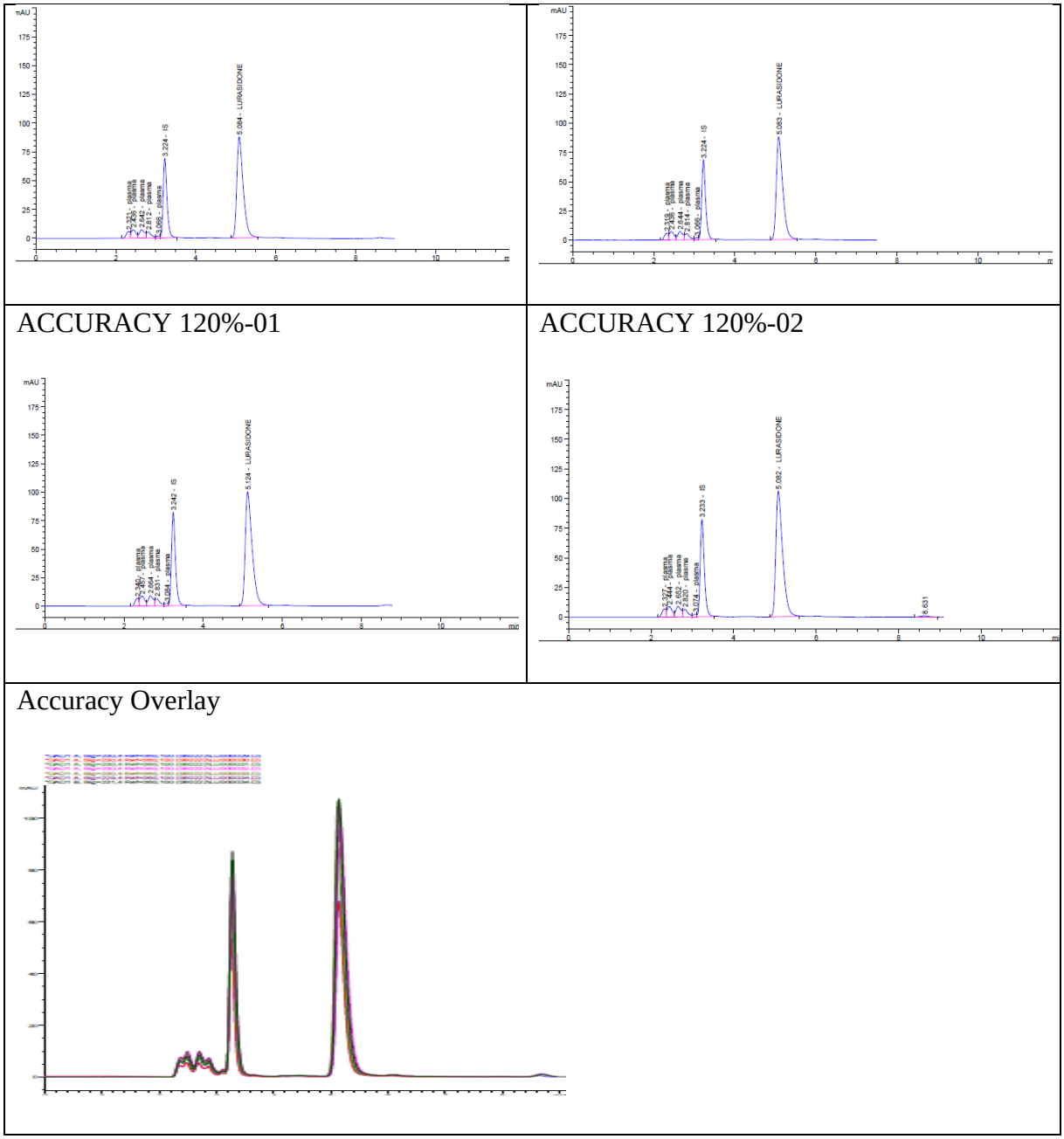


Figure 20 Accuracy chromatographs

Robustness

Robustness of method is determined by applying deliberate small changes in analysis condition. To check there is no change in retention time and chromatographic conditions. Robustness of the proposed method was done by variation of wavelength by 1nm, flow rate by 0.1min/ml and mobile phase composition, each test was repeated at two levels. The RSD was less than 2% in each of these conditions indicating that method is robust. Table 24,25,26 shows robustness data and Figure 21,22,23 shows related Chromatographs with change in mobile phase, flow rate and wavelength respectively.

Table 24: Robustness results with variation on mobile phase concentration

Mobile phase: 64% Methanol +36% (0.05 % OPA) water			Mobile phase: 66% Methanol +34% (0.05 % OPA) water		
Sr No.	Conc. µg/mL	Area	Sr No.	Conc. µg/mL	Area
1	20	985.2151	1	20	975.8138
2	20	989.9898	2	20	977.2097
	Mean	987.6		Mean	976.51
	SD	3.38		SD	0.99
	%RSD	0.34		%RSD	0.10

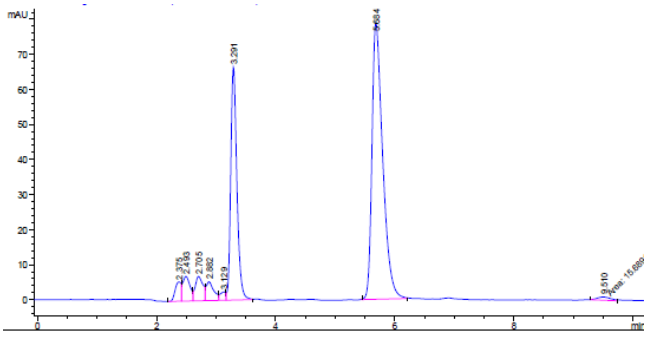
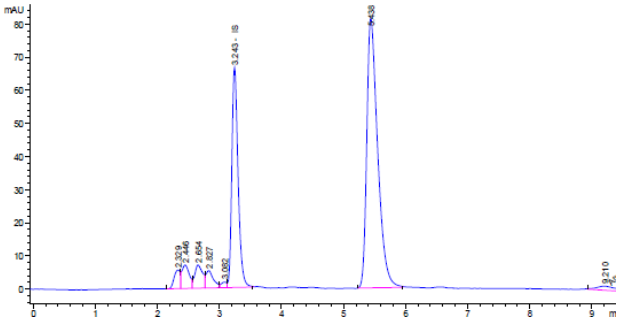
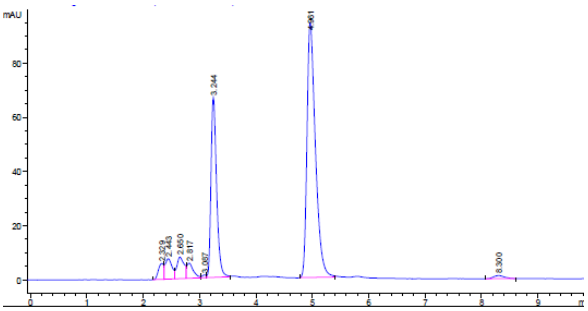
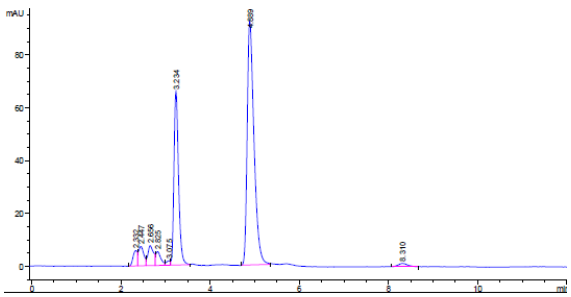
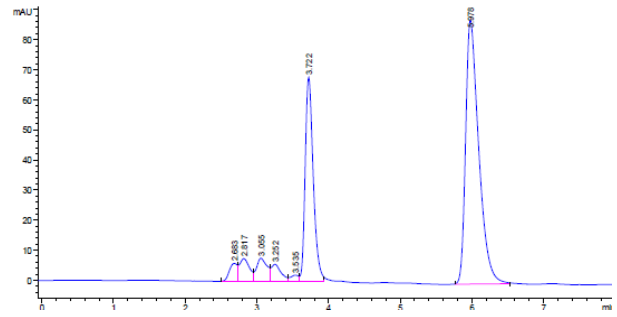
ROUBUSTNESS COMP CHANGE 64 M+36 WATER (20 MCG-01)	ROUBUSTNESS COMP CHANGE 64 M+36 WATER (20 MCG-)02
	
ROUBUSTNESS COMP CHANGE 66 M+34 WATER (20 MCG-)01	ROUBUSTNESS COMP CHANGE 66 M+34 WATER (20 MCG-)02
	

Figure 21: Chromatographs with change in mobile phase

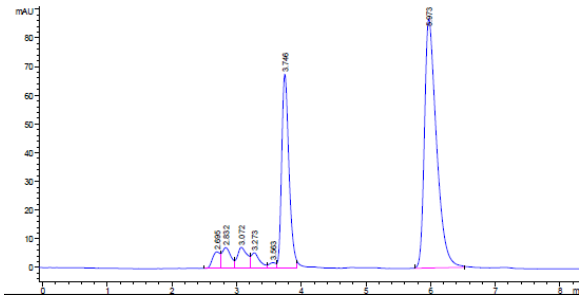
Table 25: Robustness results with change in flow rate

FLOW RATE-0.7 mL/min			FLOW RATE-0.9 mL/min		
Sr No.	Conc. µg/mL	Area	Sr No.	Conc. µg/mL	Conc. µgm/mL
1	20	1115.0247	1	20	863.3429
2	20	1115.7822	2	20	870.3416
	Mean	1115.40		Mean	866.84
	SD	0.54		SD	4.95
	%RSD	0.05		%RSD	0.57

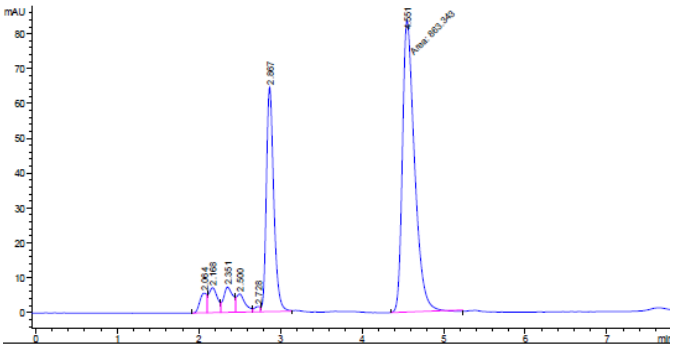
ROBUSTNESS flow 0.7 ml/min-01



ROBUSTNESS flow 0.7 ml/min-02



ROBUSTNESS flow 0.9 ml/min-01



ROBUSTNESS flow 0.9 ml/min-02

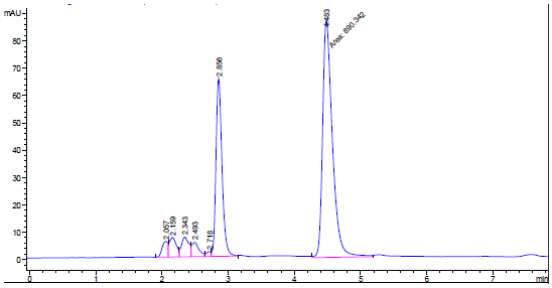


Figure 22: Chromatographs with change in flow rate

Table26: Robustness results with change in wavelength

WAVELENGTH: 229 NM			WAVELENGTH:231 NM		
Sr No.	Conc. µg/mL	Area	Sr No.	Conc. µg/mL	Area
1	20	1234.5845	1	20	1084.598
2	20	1230.5487	2	20	1085.36214
	Mean	1232.6		Mean	1084.98
	SD	2.85		SD	0.54
	%RSD	0.23		%RSD	0.05

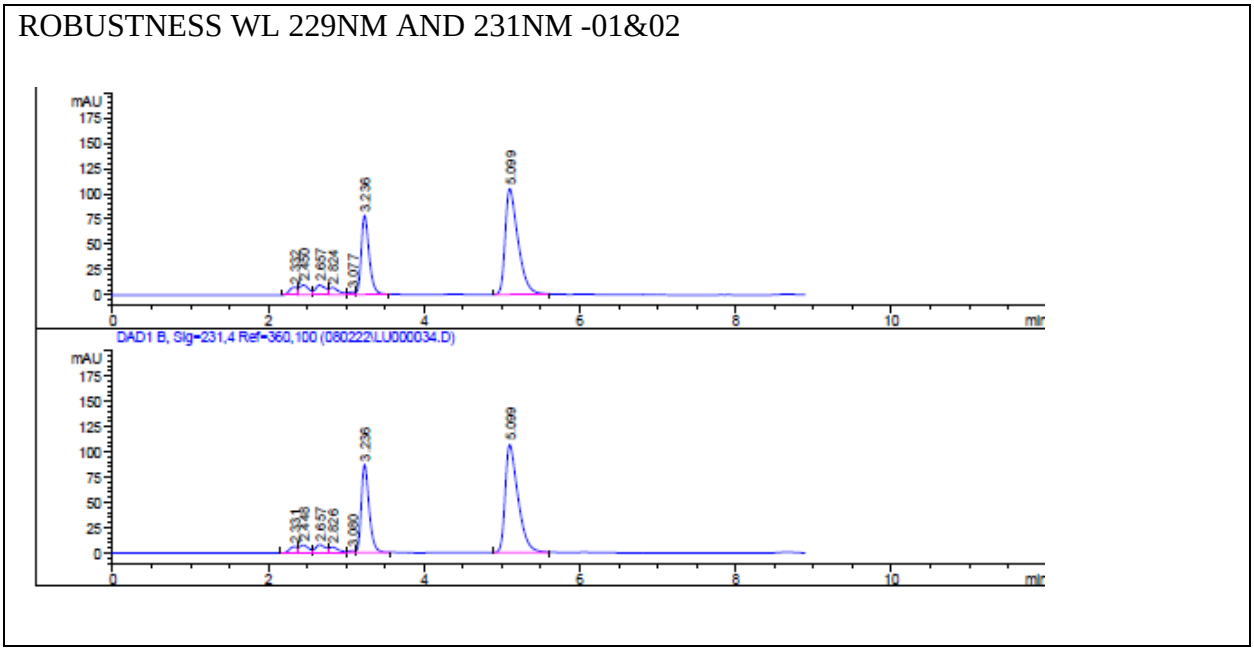


Figure 23: Robustness chromatograms with change in wavelength

Conclusion

LH-NLC consisting of GMS, Capryol-90 & Tween-80. LH-NLC was prepared by hot emulsification method

The individual effects of CPP on CQA were demonstrated using three-factor three level Box Behnken experimental Design (BBD) with the objective of achieving desired product quality. Nanostructured lipid carriers (NLCs) showed improved entrapment efficiency and drug release. This was further supported by surface morphology results which showed that LH-NLC uniform morphology.

Pharmacokinetics and brain distribution studies shows statistically significantly increased concentration of LH-NLC in plasma and in brain than plain LH.

Hence, it can be concluded that NLC based carrier system developed with enhanced quality aspects can be successfully using QbD approach which will provide effective delivery system for delivery of LH in the treatment of psychosis.

Next actions planned to be completed by February 2023

Data to be compiled for below

- Ex-vivo and histopathological study
- Invitro study
- Serum stability and Freeze thaw stability

Thesis writing : First draft is given to the guide with available data.

Research paper: Main research project paper to be communicated with above studies data.

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